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DELAYED GERMINATION IN SEED OF ALISMA PLANTAGO

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 194

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(WITH EIGHT FIGURES)

I. Introduction

The cause of delay in the germination of seeds of various water plants has been the subject of considerable discussion and some experimentation. MÜLLER (**23**) early found that seeds of *Eichhornia* and *Heteranthera* do not germinate without first being desiccated, and LUDWIG (**26**) demonstrated this for seeds of *Mayaca fluviatilis*. PFEFFER (**23**) says, "nor is it certain whether BROWN and MÜLLER are correct in assuming that previous drying is essential for the further development of *Chlamydomonas* or for the germination of the seeds mentioned." A. FISCHER (**12**) found that acids and bases arouse many seeds of water plants from their dormancy, and attributes the action of these reagents to an embryo rather than a coat effect. The following species were studied by FISCHER: *Alisma Plantago*, *Potamogeton lucens*, *P. pectinatus*, *Hippurus vulgaris*, *Polygonum amphibium*, *Scirpus lacustris*, *S. maritimus*, *Sagittaria platyphylla*, *S. sagittifolia*, and *Sparganium ramosum*.

Somewhat later, CROCKER (**6**) cast doubt upon FISCHER'S conclusion that the acids and bases are effective through their action on the embryos. It was found that the seeds of various

water plants (*Alisma*, *Sagittaria*, *Eichhornia*, etc.) germinate readily in distilled water frequently changed, provided the seed coats are broken or removed with care not to injure the embryo. From this it would seem that the embryos are not dormant if the limiting effects of the coats are not present. EWART (11) confirms these results by finding that the seeds of *Sagittaria* germinate readily after abrasion with sandpaper. He also points out the fact that the effect of the acid or the base is not counteracted by the application of a sufficient amount of the other to neutralize it. This criticism is especially significant in the light of the finding reported later in this paper, that the coat is largely composed of pectic substances.

It is evident that the dormancy in the seeds of water plants is of a different type from that in the seeds of the haw (9, 10), apple, peach, etc. In these forms the naked embryos do not grow when they are supplied with all ordinary germinative conditions if they are not first after-ripened. OSTENFELD (22) finds, confirmatory of the much earlier work of GUPPY (15), that the passage through the alimentary canals of birds greatly improves the germination of various water seeds. He considers further work necessary to justify the conclusion of either FISCHER or CROCKER. This statement seems to be fully justifiable, for it is possible that the reagents used by FISCHER have their total effect through their joint action on coats and embryos, although the latter when coat-free are certainly not dormant. It is for the purpose of throwing more light on this question that the following experiments are reported. Up to date the work has been entirely limited to a study of the seeds of *Alisma Plantago*. Of forcing agents, hydrochloric acid mainly has been studied.

II. Material

The ripened fruit of *Alisma Plantago* as it is shed from the plant is an akene, from which the pericarp can be removed by slight rubbing of the fruit between the fingers. This structure seems to play no rôle in water-intake or delay in germination, hence, for convenience in detecting the quality of the seed and for avoiding errors due to the large inert mass, the fruits worked with were

always freed from this structure, leaving only the seed proper. A single harvest of seeds thus prepared shows much variation between the individuals both in appearance and in physiological behavior. In color the seeds vary from a deep reddish brown to a light pink. As a general rule the intact dark seeds absorb water less rapidly and to much slighter total amount than the light-colored ones. With the coats broken or removed, they also show far less vigor in water absorption and in growth rate. For this reason, in the experiments here reported, light-colored seeds of plump appearance were selected chiefly. Even with this precaution to remove less vigorous ones, the seeds showed great variation in rate of water absorption and of growth.

The air-dry seeds freed from the pericarp retain a relatively large percentage of water. The amount approximates 16 per cent, varying with the humidity of the air and with the seed. The large percentage of water retained in contrast to many fatty seeds is probably related to the richness of the embryo in starch and of its walls in pectic substances, and to the high pectic content of the relatively thick coat.

When seeds were to be used with coats broken or partly removed, it was at first done by inserting a thin knife blade between the two arms of the embryo. This method did not prove desirable, for it left many coats intact, only splitting the two layers lying against the embryo from each other but not breaking them. It also often cracked the embryos at the arch. It was soon found that slight pressure of a knife blade at the micropyle end removed a quite definite cap of the coat from that end of the embryo. The break occurs at thin regions well shown in fig. 1. In some experiments, the coats were also removed by similar means from the end of the embryo adjacent to the micropyle. In this case the breaking was somewhat more irregular, and the size of the pieces showed more variation, although at this end of the embryo the coat shows very definite thinner places at the position of the cross-walls, as is the case at the micropyle end. The feature just described also accounts for the method by which the embryo breaks the coat after the seed is treated with an acid or a base. In this case the same sort of cap is pushed off the micropyle end of the coat by the lengthening

embryo. Before embryos from which a portion of the coat was removed were used, they were examined with the microscope to see that no injury was incurred in the process.

All the cultures were made under water or other solutions mentioned. The seeds will not grow normally between filter papers or on wet absorbent cotton, probably owing to insufficient water supply.

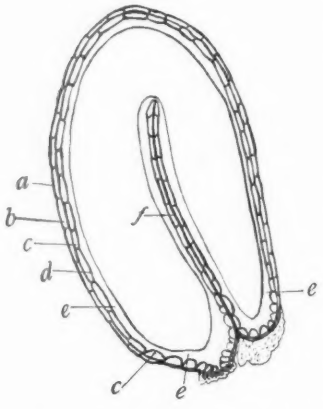

FIG. 1.—Camera drawing of a longitudinal section of the seed coat of *Alisma Plantago: a*, outer wall of the outer layer of cells, pectic substances swelling up and slowly dissolving in water; *b*, contents and cross-walls of the outer layer of cells, largely pectic material; *c*, contents and cross-walls of the inner layer of cells, largely pectic material; *d*, inner wall of inner layer of cells slightly suberized; *e*, acellular inner layer of coat, possibly originating from the endosperm, a mixture of pectic substance and hemicellulose.

III. Experiments

A number of experiments showed that the embryos themselves have no period of dormancy or need of after-ripening, for fruits just gathered from the plants gave prompt germination when the seed coats were ruptured and the seeds placed in water. Likewise, acid or base-treated seeds never showed as high percentage of germination (within 10–30 per cent) as seeds from which a portion of the coat was carefully removed. In short, there is no evidence of stimulation by the acid and base; they are simply more or less efficient agents for removing an obstruction to germination, never equaling in efficiency the mechanical method.

Intact seeds that have been soaked in water for 12 hours and then transferred to 2 mol. sodium chloride solution show rapid shrinkage, reaching a constant weight which they maintain as long as they are in the solution. These determinations are subject to rather large errors owing to the great surface represented by the 400 small seeds used in each test and the error of drying this surface to the same degree before each

weighing. The permanent shrinkage in salt solution may be due to a semipermeable membrane in the seed coat or to a change in the water-imbibing power of the colloids of the coats and perhaps of the embryo owing to the presence of the salt. A. FISCHER found that the intact fruits of *Sagittaria* were not at all injured by five days' soaking in mol. copper sulphate solution. We find that one month's soaking of intact seeds of *Alisma* (1 mol. $CuSO_4$) does not injure their germination, if they are thoroughly washed and the coats opened before they are germinated. The opened seeds grow better in 0.000001 mol. $CuSO_4$ than in distilled water, but very little in 0.001 mol. $CuSO_4$. Soaking of the intact seeds in 10 per cent $AgNO_3$ for 24 hours kills all of them. From these facts it seems probable that in *Alisma* and *Sagittaria* the seed coats bear semipermeable membranes akin to those found in *Xanthium* (**29**) and various grasses (**5, 28**).

1. STRUCTURE AND MICHROCHEMISTRY OF THE SEED COAT.—As the later experiments will show, the study of the structure and the microchemistry of the coat is much to the point. Fig. 1 is a camera drawing of a median longitudinal section of the coat. In the main the coat consists of two layers of cells: the outer yellow, red, or brown layer (*b*), and the inner colorless layer (*c*). At the micropyle end there is usually only one layer of cells. In the region where the two arms of the seed lie together, the two adjoining outer layers form a collapsed mass (*f*), while the cells of the inner layer are distinct. The single layer of cells at the micropyle end have deep indentations from the inner face at the cross-walls. As a consequence, the inner face in this region has a very undulating appearance. When the seeds germinate after acid treatment or when one picks off the coat at the micropyle end, a rather definite cap is removed. The cap seems to result from the breaking at one of the more outer of these indentations. Inside the cellular portion of the coat is an acellular lining mass of material consisting of a mixture of hemicellulose and pectic material. This layer is rather thick at the micropyle end of the embryo, and as thick or thicker at the opposite adjacent end of the embryo. In this connection it should be stated that it is common to find seeds with the outer layer of cells largely abraded lying in water in the dormant

condition. This shows that it is largely, if not entirely, the inner layer of cells of the coat, with perhaps the acellular layer, that is responsible for the failure to grow.

The methods given by TUNMANN (31) in his late work were followed in the microchemical tests. The following reactions justify the conclusions given in the description of fig. 1. After treating sections of the coat 48 hours with cuprammonia and then staining with ruthenium red, *a*, *c*, *e*, and part of *b* became red. Treatment for 48 hours with cuprammonia followed by 2 per cent ammonia dissolved all the section except *d* and part of *b*. Sudan III stained only the inner wall of the inner layer of cells. Chlorzinc iodide stained *a* violet and *e* blue. Chromic acid in 10 minutes dissolves everything except *d*, but after 16 hours *d* still remains intact. One half-hour's heating in 5 per cent hydrochloric acid dissolves a considerable part of *e*, especially at the hilum end and the small adjacent end of the coat. One-half hour's heating in 2.5 per cent sulphuric acid followed by treatment with IKI stains *e* blue; IKI alone gives no reaction in any part. Treatment with copper sulphate for 48 hours, followed by 2 per cent ammonia, dissolved the greater part of *c;* 2 per cent ammonia alone brings about no visible changes. Sections in water for 10 days showed the contents and most of the cross-walls in *c* dissolved, and the plano-convex cells at the large end swollen to about twice their former size.

These tests indicate that there is no cellulose in the coat. The blue coloration of *e* with chlorzinc iodide is probably due to the presence of hemicellulose rather than cellulose, as indicated by its insolubility in cuprammonia, its ease of dissolution by 5 per cent hydrochloric acid, and its ready transformation to amyloid by 2.5 per cent sulphuric acid.

It is evident that the coat is largely composed of pectic substances, with a suberized wall at *d* and an admixture of hemicellulose in *e*. The dissolution or marked swelling of *a*, the contents and cross-walls of *c*, and the plano-convex cells at the large end of the embryo, indicate that these are made of the type of pectic substances included under the terms pectic, parapectic, and parapectosic acids. Slight chemical transformation of these substances

may easily weaken the coat, so that the imbibing embryo can rupture it, for we shall see later that the evidence is strong that the delay is due to the swelling force of the embryo being unable to break the restraining coat. We must bear in mind also that without any chemical decomposition acids and bases may increase greatly the water-absorbing power of the hydrophilous pectic and hemicellulose substances of the seed coats, but undoubtedly chemical transformations as well as physical are brought about in the acid and base-sensitive substances of the seed coat.

Heating the seeds to 99–100° C. for 0.5 hr. in 2 per cent HCl, followed by 0.5 hr. in 2 per cent NaOH, the usual method of dissolving out the middle lamella of cell walls, dissolves away most of the coat at the large end of the embryo and causes the embryo to break the coat and elongate 200–400 per cent. All regions of the coat were modified; in some cases the walls were apparently largely dissolved away, leaving a thin lamella, and in others gelatinized and greatly swollen. Some color dissolved out of the wall in the acid, but immediately upon transfer to the base the latter took on a deep red color. Heating to 99–100° in 2 per cent NaOH for 0.5 hr. gave similar results, and 0.5 per cent NaOH was almost as effective. Even at room temperatures, similar but less marked changes appeared in 2 per cent NaOH, and at this temperature 0.5 per cent caused noticeable exosmose of coat pigments and decomposition of the coats within a half-hour.

It is found that seeds treated with 0.3 N HCl for 1 hr. at 40° C. give 30–50 per cent germination in distilled water; much poorer germination in 0.000001 mol. $CuSO_4$, and none at all in 0.001 mol. $CuSO_4$. As has been pointed out, the embryos with coats partly removed germinate better in 0.000001 mol. $CuSO_4$ than in distilled water. It seems that the copper is rather readily absorbed by the gel-like coat and the consistency of the latter changed. The absorption is evidenced by the coat turning a dark dull brown rather rapidly in the stronger solution and slowly in the weaker. The rather plastic gel of the coat becomes hard and brittle and is easily cracked under the pressure of a scalpel. The hardening takes place to a more marked degree in untreated seeds soaked in water. Apparently this hardening of the coat gel increases the

resistance of the coat to the enlarging embryo, and thereby prevents germination, even in the treated seeds. The facts reported in this paragraph show the danger of applying reagents to intact seeds and drawing conclusions from the results as to the effects the reagents have on the embryos.

2. WATER RELATIONS OF THE EMBRYO AND COAT.—In considering the water relations of the embryo and coat we will first examine the rate of absorption of water by the seeds under three different conditions: (1) seeds with the coats removed from the micropyle end of the embryo; (2) intact seeds untreated; (3) intact seeds treated with solutions of 0.2 N HCl for various periods. Of the six experiments performed only two will be given, since they

TABLE I

Showing percentage of increase over air-dry weight of *Alisma* seeds treated as follows: I, 400 seeds in water, coats broken; II, 400 seeds in water, coats intact; III, 400 seeds, coats intact, in 0.2 N HCl for 2 hours then transferred to water. Temperature 20° C.

No. of hours soaked	Percentage of increase on basis of air-dry weight		
	I	II	III
1	40	31.6	34
2	65	40	48
3	65	40	48
4	68	40	52
18	73	50	52
23	180	50	52

represent the trend of the results. Table I gives the data for the first of these, and fig. 2 presents the results in the form of curves.

Table II gives the data for the second experiment, and fig. 3 gives the results in the form of curves.

Only the general features of the curves in these experiments are of value, because of the necessary errors involved in the experiment. The seeds are very small, and the 400 used in each culture have a large specific surface. Any variation in degree of drying before weighing gives considerable error. Another variation that appeared in these experiments was the promptness with which the acid treatment became effective. This accounts for the curve for intake by the acid treatment running close to the untreated

ones in the first experiment and falling between the other two curves in the second experiment. In the first experiment very few of the embryos had broken the coat previous to the 43d hour, while

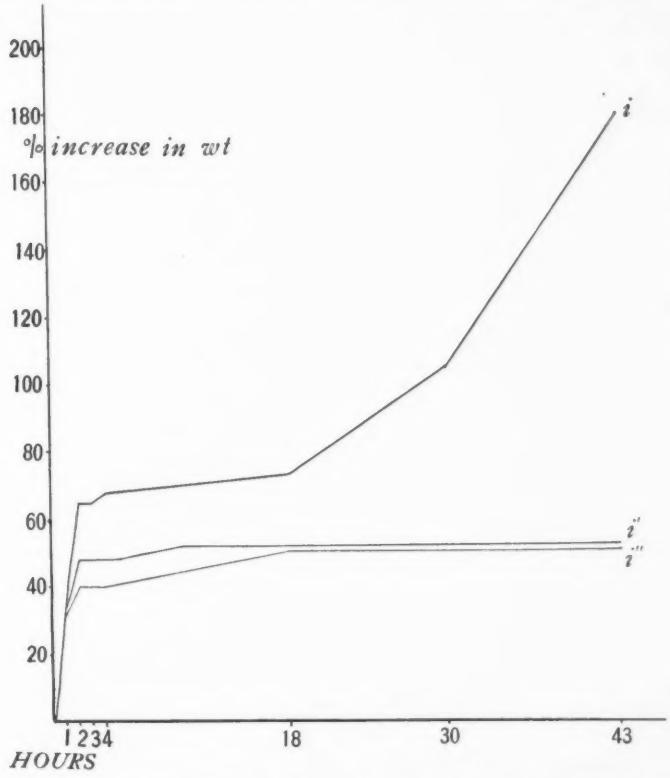

FIG. 2.—Rate of water absorption (percentage of increase over air-dry weight) by *Alisma* seeds with pericarp removed: temperature 20° C.; *i*, 400 seeds, coats broken and seeds soaked in water; *i'*, 400 seeds, coats intact, first two hours in 0.2 N HCl and rest of the time in water; *i''*, 400 seeds, coats intact and soaked in distilled water.

more than 30 per cent of them did so within 50 hours. In the second many of the embryos had broken the coats after 10 hours, and about half had broken the coats within 43 hours. The difference in the promptness of the action of the acid in these two

experiments may be explained in part by the difference in temperature. When FISCHER used 0.2 or 0.3 N HCl with one or two hours' exposure as a forcing agent, he found 40° C. a very effective temperature. Individual seeds also show great variation in the readiness with which they swell to the maximum and begin growth. Old seeds, such as those used in the experiments with $CuSO_4$, seem more resistant to HCl. This is prominent even in cases where the coats are broken, as later results will show.

The curves for the absorption of water with the coats broken correspond closely in the two experiments reported, as they do in all the experiments. In these seeds the absorption of water is very rapid during the first 2–4 hours, amounting to 65–80 per cent of the

TABLE II

Showing percentage absorption of water (figured on the basis of air-dry weight) by *Alisma* seeds: I, coats broken, seeds in water; II, coats intact, seeds in water; III, coats intact, seeds 2 hours in 0.2 N HCl and then in water. Temperature 25° C.

No. of hours soaked	Percentage of water absorbed on basis of air-dry weight		
	I	II	III
1	32.5	23	45
2	55	33	52
6	80	45	53
9	80	45	61
21	101	51	.....
32	160	51	73
52	.....	.....	96

air-dry weight, or to approximately 100 per cent of the dry weight. This is followed by a period of 10 or more hours of very slow water absorption, which in turn is followed by rapid water absorption. The first period of rapid water intake is largely a matter of imbibition and osmosis. The slow water absorption lying between these marks the interval between imbibitional and growth enlargement.

With the untreated intact seeds the water absorption essentially all occurs within 2–4 hours, and approximates 45 per cent of air-dry weight, or about 70 per cent of dry weight. The imbibitional and osmotic capacity of the coat and embryo for water is not nearly satisfied, for, as is shown above, when the coat is broken

water absorption is increased by about 50 per cent. From these facts, together with the structure of the seed, as well as from facts to be given later, one might be led to conclude that the embryo

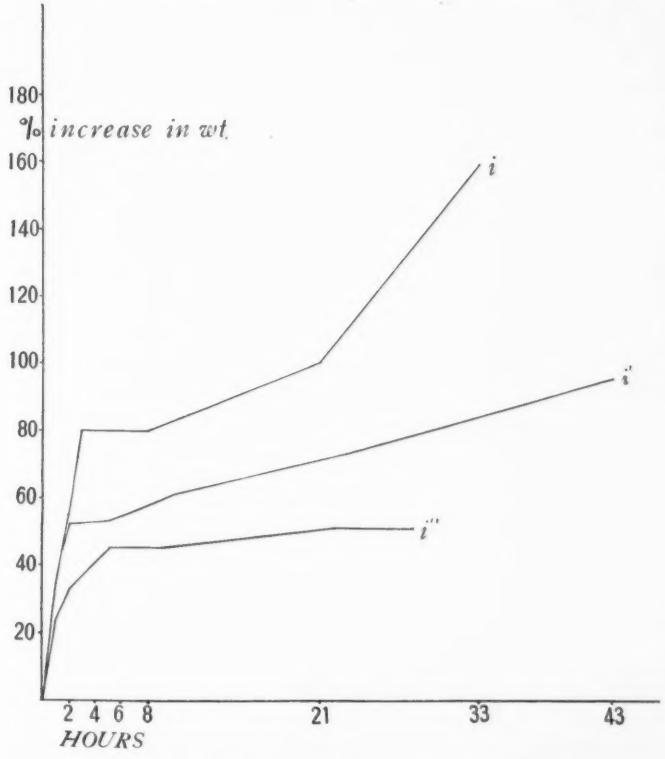

FIG. 3.—Rate of water absorption (percentage of increase over air-dry weight) by *Alisma* seeds with pericarp removed: temperature 25° C.; *i*, 400 seeds, coats broken and seeds soaked in water; *i'*, 400 seeds, coats intact, first two hours in 0.2 N HCl and rest of the time in water; *i''*, 400 seeds, coats intact and soaked in distilled water.

is hindered from swelling before its imbibitional forces are satisfied, because these forces are not sufficient to rupture the coat. The initial rate of water intake by both the intact seeds and the seeds with the coats broken equals that of the rapidly imbibing seeds of

Xanthium as shown by SHULL (**29**), and far exceeds that of the slow imbibing seeds of the grasses as found by SCHRÖDER (**28**). In turn this rate of water intake is considerably exceeded by some of the Cucurbitaceae (**32**) (*Cucurbita argyrosperma*), where, however, the intake is largely due to a spongy capillary layer in the coat itself. On the other hand, we do not know to what degree the water intake in intact *Alisma* seeds is due to the coats; it is certainly due to this to a very large extent.

3. EFFECT OF ACIDS UPON RATE OF WATER INTAKE BY THE EMBRYOS.—MARTIN FISCHER (**13**) has found that bases and acids increase the rate as well as total amount of water absorption by colloids. BOROWIKOW (**4**) has shown that acids increase the rate of growth, or at least the rate of elongation of roots in water; while PROMSY (**25**) and ECKERSON (**10**) have shown that acids hasten the rate of germination of many seeds. These workers attribute the effect of the acids, at least in part, to the fact that they further the hydration of the colloids—in the case of the plant organs, the cell colloids. It will suggest itself immediately that the effect of the acids on the germination of water seeds may be due to their increasing of the imbibition force of the embryo, thereby enabling the embryo to rupture the coat. If this proves to be the case, the interesting situation appears that while the coat is the real cause of the delay, the effect of the acid is upon the embryo itself, increasing its imbibition power and enabling it to rupture the restraining coat. To determine this point experiments were made to find the rate of elongation of the embryo in water and in solutions of hydrochloric acid and sodium hydrate of various concentrations. In order to allow free access of the acid solutions and water to the embryo, the coat was removed at both ends of the embryo as described under "material." Only embryos that remained uninjured after the removal of the coats were used in the experiments. The first experiment was run with water, 0.01 N HCl, and 0.001 N HCl. Table III shows the rate of elongation of 20 embryos placed in water and measured at suitable intervals for 96 hours.

A great variation in the rate of elongation of the various embryos is shown. This seems to be characteristic of every crop gathered. Nos. 6, 13, and 20 are individuals showing low rate of elongation

TABLE III

Embryo	Length of embryo	Time in water and length of embryo (coats broken)						
	Dry	1 hr.	3 hrs.	6½ hrs.	23 hrs.	31 hrs.	50 hrs	96 hrs.
1	95.5	104	112	117	140	140.5	145	360
2	100	115	121	125	138	141	152	396
3	95	105	117	126	146	146	148	230
4	97	103	117	121	121	122	124	161
5	87	96	114	128	142	142	148	200
6	96	101	114	128	141	138	146	147
7	99	117	127	132	151	151	157	800
8	99	109	118	129	168	199	230	430
9	89	107	111	124	137	134	134	360
10	85.5	89	99	107	113	113	116	165
11	90	104	118	116	116	115	120	230
12	94	101	117	129	143	143	170	560
13	86	106	116	119	143	144	146	144
14	93	95	118	124	134	140	157	570
15	84	114	122	121	121	123	124	430
16	82	89	101	103	114	136	139	180
17	74	88	97	106	118	116	120	270
18	100	111	125	141	185	210	276	540
19	83	190	116	124	132	132	136	400
20	95	111	122	124	132	134	136	140

TABLE IV

Embryo	Length of embryo	Time in 0.01 N HCl and length of embryo (coats broken)						
	Dry	1 hr.	3 hrs.	6½ hrs.	23 hrs.	31 hrs.	50 hrs.	96 hrs.
1	92	114	114	112	120	.....	122	.....
2	84.5	110	115	124	124	.....	129	.....
3	103	106	113	121	120	.....	123	.....
4	95.5	117	116	129	124	.....	134	.....
5	91.5	104	112	124	126	.....	126	.....
6	102	115	120	135	130	.....	162	.....
7	91	100	102	112	112	.....	111	.....
8	77.5	96	106	109	108	.....	108	.....
9	75.5	98	95	106	106	.....	105	.....
10	102	103	120	130	131	.....	131	.....
11	89.5	97	105	115	116	.....	126	.....
12	102	125	129	134	134	.....	134	.....
13	93	110	116	120	135	.....	130	.....
14	86	95	103	127	122	.....	122	.....
15	86	96	105	111	115	.....	112	.....
16	102	113	121	137	132	.....	132	.....
17	91	110	115	125	124	.....	127	.....
18	101	111	93	120	117	.....	135	.....
19	106	111	121	139	132	.....	138	.....
20	100.5	105	112	121	119	.....	122	.....

TABLE V

Embryo	Length of embryo	Time in 0.001 N HCl and length of embryo (coats broken)						
	Dry	1 hr.	3 hrs.	6½ hrs.	23 hrs.	31 hrs.	50 hrs.	96 hrs.
1	83	99	100	108	103	.....	106	106
2	108.5	188	132	140	149	.....	256	350
3	85.5	103	108	112	127	.....	129	145
4	101	96	118	131	150	.....	160	180
5	92	109	120	125	120	.....	122	125
6	104.5	125	129	134	134	.....	141	157
7	98.5	107	115	122	130	.....	130	144
8	81	98	101	109	112	.....	111	111
9	87	101	109	120	125	.....	125	125
10	96.5	110	110	118	118	.....	118	121
11	98	125	127	132	131	.....	132	137
12	93	101	115	126	131	.....	131	129
13	90	112	125	129	126	.....	128	125
14	98	105	115	134	136	.....	235	290
15	87.5	98	114	110	114	.....	119	110
16	112	122	134	145	148	.....	156	192
17	85.5	95	111	119	122	.....	122	156
18	105	115	125	135	134	.....	149	200
19	96	101	114	123	120	.....	121	127
20	75.5	84	95	104	98	.....	98	98

TABLE VI

Showing the elongation of 20 *Alisma* embryos each in water, 0.01 N HCl, and 0.001 N HCl. Coats removed at both ends of the embryo. Temperature 25° C.

Condition of the embryos	Total length of embryos	Average length of embryos	Percentage of increase over dry length
Air dry	1834.5	91.7	.....
1 hour in water	2073.5	103.6	13.1
3 hours " "	2312.5	115.6	26.1
6⅓ " " "	2470.0	123.5	34.7
23 " " "	2759.5	137.9	50.4
31 " " "	2832.0	141.6	54.3
50 " " "	3025.0	151.2	64.9
96 " " "	6720.0	336.0	266.0
Air dry	1876.0	93.8	.....
1 hour in 0.01 N HCl	2104.0	105.2	12.2
1 hour in 0.001 N HCl	2257.5	112.9	20.3
6⅓ " " " "	2471.0	123.5	31.2+
23 " " " "	2462.0	123.1	31.2
50 " " " "	2512.0	125.6	33.9
	No further elongation		
Air dry	1878.0	93.9	.....
1 hour in 0.001 N HCl	2131.0	106.5	13.4
3 hours " " "	2319.0	116.0	23.5
6⅓ " " " "	2474.0	123.7	31.7
23 " " " "	2515.0	125.7	33.9
51 " " " "	2789.5	139.5	48.5
96 " " " "	3139.0	156.9	67.1

and a low total at the end of 96 hours, while nos. 7, 22, and 14 show both a high rate and a high total. Table IV shows the elongation of 20 embryos in 0.01 N HCl; and table V the elongation of 20 embryos in 0.001 N HCl.

Table VI summarizes the results for the 20 seeds each in water, 0.01 N HCl, and 0.001 N HCl, and fig. 4 presents the results in the form of curves. The percentage of elongation is platted on the abscissa and the time on the ordinate. It is evident from these

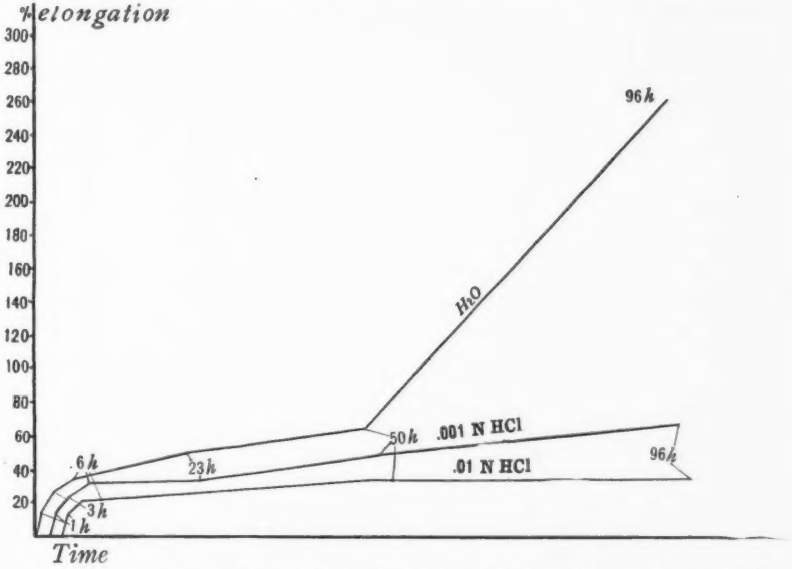

FIG. 4.—Rate of elongation of *Alisma Plantago* embryos in water, 0.01 N HCl, and 0.001 N HCl.

curves that the rate of elongation in water equals or exceeds that in either solution at all times, and shows a great advantage over both from 50 hours on. In the 0.001 N HCl cultures, the rate of elongation and the total elongation stands between that of water cultures and the 0.01 N HCl cultures.

A second experiment was performed using water, N/3000, and N/6000 HCl. These results are summarized in table VII and put in the form of curves in fig. 5. In this case the N/3000 HCl at

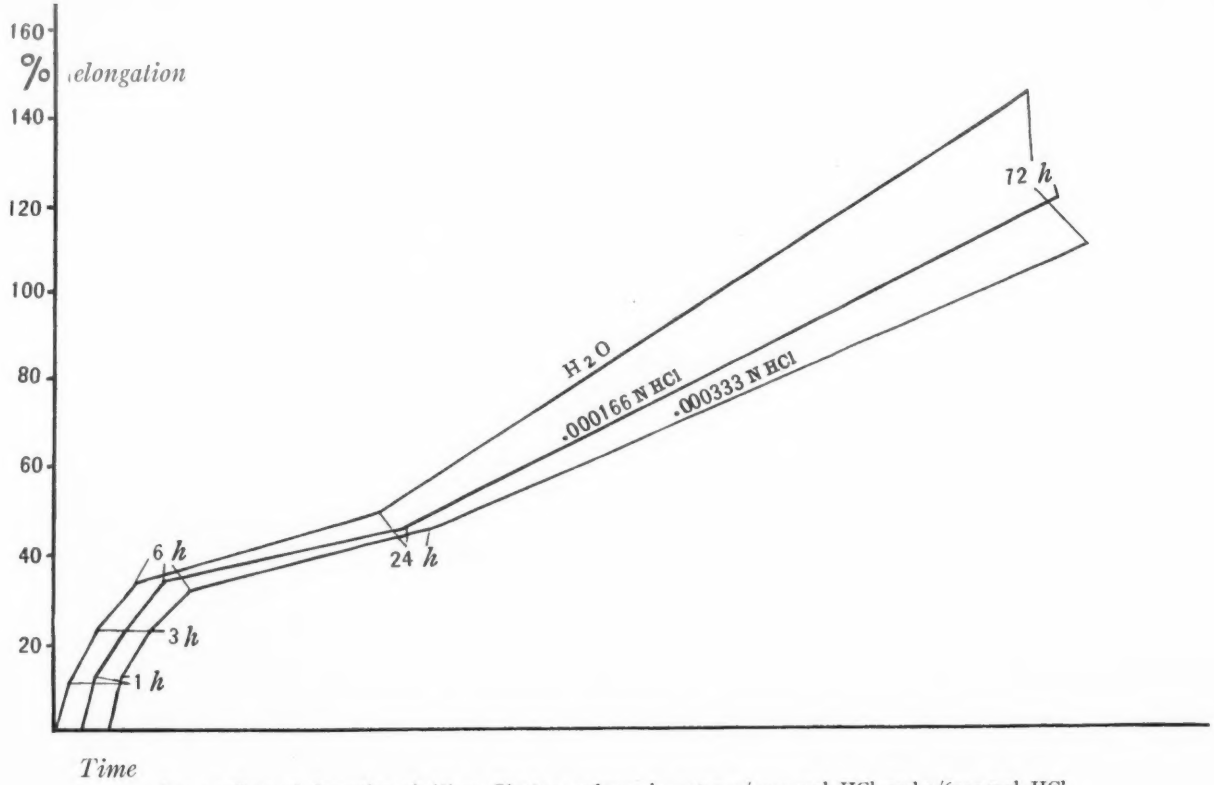

FIG. 5.—Rate of elongation of *Alisma Plantago* embryos in water, 1/3000 mol. HCl, and 1/6000 mol. HCl

first slightly leads water in rate of elongation, but falls behind it later. This slight early advantage can well be accounted for by the variation in the embryos from employment of so few, while the later falling behind water is undoubtedly related to injurious effects of the acid. The N/6000 HCl at first kept pace with water but later fell behind it.

TABLE VII

Showing the elongation of *Alisma Plantago* embryos in water, 1/3000 mol. HCl, and 1/6000 mol. HCl.

Condition of the embryos	Total length	Average length	Percentage of increase over air-dry length
Air dry	811	101.4	
1 hour in water	899	112.5	10.5
3 hours " "	996	124.5	22.8
6 " " "	1080	135.0	33.2
24 " " "	1208	151.0	49.0
72 " " "	1967	246.0	142.5
Air dry	817	102.0	
1 hour in N/3000 HCl	918	114.7	12.3
3 hours " " "	1035	117.0	26.6
6 " " " "	1102	137.7	35.0
24 " " " "	1193	148.9	45.3
72 " " " "	1788	223.5	119.0
Air dry	1005	100.5	
1 hour in N/6000 HCl	1114	111.4	10.8
3 hours " " "	1222	122.2	21.5
6 " " " "	1312	131.2	30.5
24 " " " "	1449	144.9	44.1
72 " " " "	2107	210.7	109.0

Table VII A summarizes two other sets of measurements on the effect of N/3000 and N/6000 HCl on the rate of elongation.

TABLE VII A

Average percentage elongation for 10 embryos each in water and the HCl solution mentioned.

	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.
N/3000 HCl	10.1	16.9	24.7	28.9	32.0
N/6000 HCl	10.8	16.9	23.0	27.0	30.0
Water	9.1	16.2	21.9	27.5	30.6
N/3000 HCl	11.0	20.0	26.0	29.0	32.0
Water	10.0	16.8	24.7	28.7	31.0

From all these measurements it seems barely possible that acid solutions hasten somewhat the rate of elongation of the embryo, and that the maximum effect appears at about N/3000 in a 5-hour period, but the effect is not great.

Table VII B shows the effect of various concentrations of NaOH on the rate of elongation of embryos. The solutions were protected from CO_2 of the air, but of course were continually being transformed to carbonates by the CO_2 from the respiring seedlings, so the concentration of the hydrate was falling from the beginning of the experiments.

TABLE VII B

Average percentage of elongation for 10 embryos each in water and the NaOH solutions mentioned.

	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	20 hrs.
0.01 N NaOH	12.9	17.8	23.5	28.2	30.5	43.0
0.001 N NaOH	12.7	19.2	24.9	32.6	35.7	49.0
Water	10.2	16.5	22.6	27.0	30.3	42.9
0.0001 N NaOH	11.9	18.9	25.0	30.7	34.7	46.9
N/3000 NaOH	11.9	18.0	24.2	27.6	30.4	40.3
Water	10.4	17.3	23.2	28.0	30.1	39.0

In each case the results in the various solutions should be compared only with their corresponding checks in water; also the checks in water are not comparable with each other, because the various experiments were not run at the same temperatures.

The base seems to increase imbibition rate very noticeably in contrast to acid, and there seems to be a distinct optimum concentration which for a 20-hour period lies in the region of 0.001 N NaOH. Such an optimum appears for the swelling of various colloid gels in the case of both acids and bases.

There is then doubtful evidence for acids causing increased rate of elongation of the embryo, and very certain evidence for bases doing so. It is probable, but by no means established, that along with such an increase in rate of elongation there goes an increase in force of elongation. If this be so, the base and perhaps the acid may further the breaking of the coat in part by an effect on the embryo.

4. TURGOR IMBIBITION AND GROWTH ELONGATION OF THE EMBRYOS.—The object of the experiments reported in this section

is to see to what extent the elongation of the embryos in water is a matter of imbibition and osmosis, and to what extent a matter of growth. Embryos with the seed coats removed at both ends were measured and placed in water for various lengths of time and the elongation determined. They were then transferred to plasmolytic agents and the amount of shrinkage determined. Cane sugar was finally chosen as the water-absorbing agent, for it produced permanent shrinkage and showed relatively low toxicity. Embryos which have been soaked in water for 12 hours and then transferred to 2 volume mol. cane sugar for 24 hours are still capable of normal growth when transferred to water. On the other hand, lithium chloride, which in saturated solution has great water-absorbing power, proved unsatisfactory both because of its toxicity and the variation in results.

Table VIII shows the average change in length caused in 8 embryos by soaking them 2.5 hours in water and then transferring them to 2 volume mol. sugar solution for 40 hours.

TABLE VIII

Elongation of 8 *Alisma* embryos soaked 2.5 hours in water, then transferred to 2 volume mol. cane sugar solution. Seed coats removed from both ends of the embryo. Temperature 25° C.

Treatment of embryos	Total length of embryos	Average length of embryos	Percentage of elongation
Air dry	814.0	101.8	
2.5 hours in water	969.5	121.2	19.1
5 " " sugar solution	866.0	108.2	6.4
25 " " " "	856.0	107.0	5.2
40 " " " "	840.0	105.0	3.7
50 " " " "	840.0	105.0	3.7
Extreme individuals—		Length of embryo	
1. Dry		109	
2.5 hours in water		139	27.5
40 " " sugar solution		111	1.9
2. Dry		96	
2.5 hours in water		108	12.5
25 " " sugar solution		98	2.0
40 " " " "		98	2.0

Two and one-half hours in water gives an elongation of 19 per cent of the air-dry length, while the transfer to the sugar solution shrinks the embryos back to within 3.7 per cent of the air-dry

length. The two extreme individuals are also given in the table. One showed elongation in water of 27.5 per cent and shrinkage in the sugar solution back to 1.9 per cent; the other extreme individual showed an elongation of 12.5 per cent in water and shrinkage back to 2 per cent in the sugar solution. In all the measurements of this section we find great variation between the individual embryos.

Table IX shows the elongation of 10 air-dry embryos, freed from the seed coats at both ends, when they are placed in 2 volume mol. cane sugar.

TABLE IX

Elongation of 10 *Alisma* embryos placed in 2 mol. cane sugar solution for 52 hours. Seed coats removed at both ends of the embryos.

Treatment of embryos	Total length of embryos	Average length of embryos	Percentage of elongation
Air dry	1080.0	108.0	
5 hours in sugar solution	1093.0	109.3−	1.2+
20 " " " "	1109.7	111.0−	2.7+
40 " " " "	1113.5	111.3+	3.1
52 " " " "	1114.5	111.4+	3.2

After 52 hours these embryos showed an average elongation of 3.2 per cent. Allowing for considerable variation in individuals, it is evident that about the same final elongation results whether air-dry embryos are soaked directly in 2 mol. cane sugar or whether they are first soaked in water for 2.5 hours and then transferred to the sugar solution. The 19 per cent elongation of the embryo which occurs in water in the course of 2.5 hours is all a matter of imbibition and turgor. The intact seed itself, as we shall see in the next section, elongates on the average 10 per cent when it is soaked in water for a similar period. It is probable that the embryo in the intact seed thus soaked elongates far less than 10 per cent, for the acellular mass at both ends of the embryo and at the arch show much swelling in water, as do the other layers of the coats. Were the embryos in the intact seed allowed to carry on their imbibitional and osmotic intake of water freely and break off the coat cap at the large end of the embryo, within 2.5 hours the embryos would extend 10 per cent of their total length beyond

the coat. Since the embryo has two arms of about equal length, the imbibitional and osmotic swelling would cause the embryo to extend 20 per cent the length of the seed beyond the limits of the coat. The rapid elongation of the freed embryos is brought about in part by the rapid swelling of the pectic walls. *These facts force one to believe that delay in germination in the Alisma seed is caused by the seed coat preventing the complete imbibitional and osmotic elongation of the embryo.* If one assumes that, on the average, the embryo in the soaked intact seed has elongated only 3–4 per cent of its air-dry length, and this is probably not far from correct, one sees that in the soaked condition of the seed the embryo is in water equilibrium with 2 volume mol. cane sugar solution. Such a solution has an osmotic pressure exceeding 100 atmospheres according to later estimates (**24, 27**). This probably gives a rough estimate of the force with which the embryo is pressing against the coat in the intact seed lying in water.

TABLE X

Elongation of 8 *Alisma* embryos soaked in water 5 hours and then transferred to 2 volume mol. cane sugar solution. Seed coats removed from both ends of the embryo. Temperature 25° C.

Treatment of embryos	Total length of embryos	Average length of embryos	Percentage of elongation
Air dry	803	100.4	
5 hours in water	1050	131.2	30.8
5 hours in sugar solution	879	109.9	9.5
25 " " " "	861	107.6	7.2
40 " " " "	863	107.9	7.5
Extreme individuals—		Length of embryo	
1. Air dry		97.0	
5 hours in water		131.2	40.2
5 " " sugar solution		116.0	19.5
25 " " " "		114.0	17.5
2. Air dry		103.0	
5 hours in water		128.0	24.2
5 " " sugar solution		110.0	6.8
25 " " " "		111.0	7.7

Table X shows the changes in length brought about in 8 embryos by soaking them 5 hours in water and then transferring them to 2 volume mol. cane sugar for 40 hours. Five hours in water gives an average elongation of 30.8 per cent, and in the extreme individuals

40.2 per cent and 24.2 per cent. Transfer to the sugar solution caused an average shrinkage back to 7.2 per cent elongation.

In the extreme individuals the shrinkage was back to 17.5 per cent and 7.7 per cent elongation. It is evident that permanent elongation or growth has set in to a slight but measurable degree at the end of 5 hours. In one of the extreme individuals the growth was considerable. This is another example of the great individual variation that is always evident. Fig. 6 shows these results in the form of curves.

Table XI shows the changes in length brought about in 14 *Alisma* embryos by soaking them 16 hours in water and then transferring to 2 volume mol. cane sugar for 25 hours.

In this case the average elongation in water is 36.1 per cent,

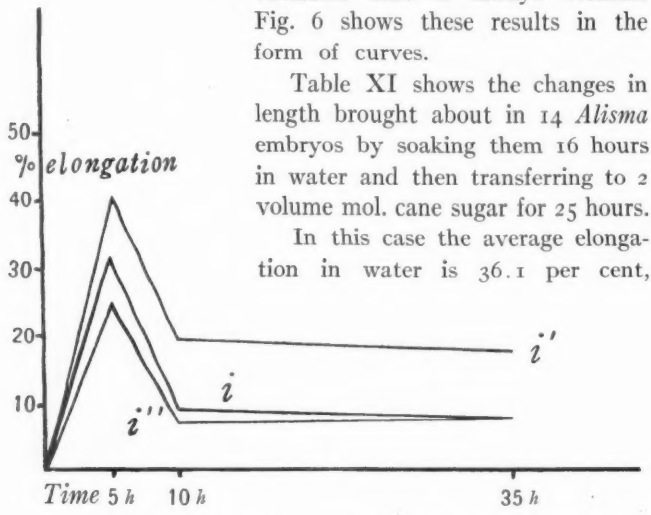

FIG. 6.—Percentage of elongation of *Alisma* embryos (seed coat removed at both ends) soaked in water 5 hours, then placed in 2 mol. cane sugar solution: measurements were made on dry embryos, after 5 hours in water, and after 5 and 30 hours in the sugar solution; *i*, curve for the 8 embryos studied; *i'*, curve for the individual showing the greatest swelling; *i''*, curve for the individual showing least swelling.

while the sugar causes a shrinkage back to 11 or 12 per cent elongation. The behavior of the extreme individuals is also shown in this table. Greater permanent elongation is evident than in the embryos in water for 5 hours. Here also there is some evidence of a slight permeability to sugar as shown by a slight swelling from 5 hours to 10 hours. Fig. 7 shows these data in the form of curves.

TABLE XI

Elongation of 14 *Alisma* embryos soaked in water 16 hours and then transferred to 2 volume mol. cane sugar solution. Seed coats removed from both ends of the embryos. Temperature 25° C.

Treatment of embryos	Total length of embryos	Average length of embryos	Percentage of elongation
Air dry	1378	98.4	
16 hours in water	1876	134.0	36.1
5 " " sugar solution	1536	109.6	11.3
25 " " " "	1543	110.2	11.9
Extreme individuals—		Length of embryo	
1. Air dry		98.5	
16 hours in water		149.0	51.0
5 " " sugar solution		115.0	16.7
25 " " " "		115.0	16.7
2. Air dry		102.0	
16 hours in water		129.0	26.4
5 " " sugar solution		105.0	3.0
25 " " " "		108.0	5.0

Table XII shows the behavior of 13 *Alisma* embryos kept in water 16 hours and then transferred to 1 volume mol. cane sugar.

TABLE XII

Elongation of 13 *Alisma* embryos placed in water 16 hours and then transferred to 1 volume mol. cane sugar for 25 hours. Seed coats removed from both ends of the embryo. Temperature 25° C.

Treatment of embryos	Total length of embryos	Average length of embryos	Percentage of elongation
Air dry	1283.0	98.7	
16 hours in water	1716.0	132.0	33.7
5 " " sugar solution	1535.0	118.0	19.0
25 " " " "	1565.5	120.4	22.0
Extreme individuals—		Length of embryo	
1. Air dry		87.0	
16 hours in water		138.0	58.5
2 " " sugar solution		109.0	25.3
2. Air dry		94.0	
16 hours in water		115.0	22.3
25 " " sugar solution		109.0	16.0

The 1 mol. cane sugar shows far less shrinkage than the 2 mol. Fig. 8 shows these data in the form of curves.

This series of experiments shows that the imbibition and osmotic forces alone cause an average elongation of about 20 per cent in the embryos partly freed from the coat. The elongation all occurs within 2.5 hours when the temperature is maintained at 25° C. This elongation of the embryo is probably three to five times that taking place when the intact seed is soaked for the same time or even for months. It is probable that the swelling of the embryo in thickness is inhibited to quite as large a percentage by the coat, for when one observes the embryo protruding from the coat, owing to the removal of the cap at either end, he sees a marked constriction at the line of the broken edge of the coat. The much swollen acellular layer seems to restrict greatly the swelling of the portion of the embryo within it. Growth begins within five hours on the average when partly naked embryos are placed in water at 25° C. Even 16 hours in water at this

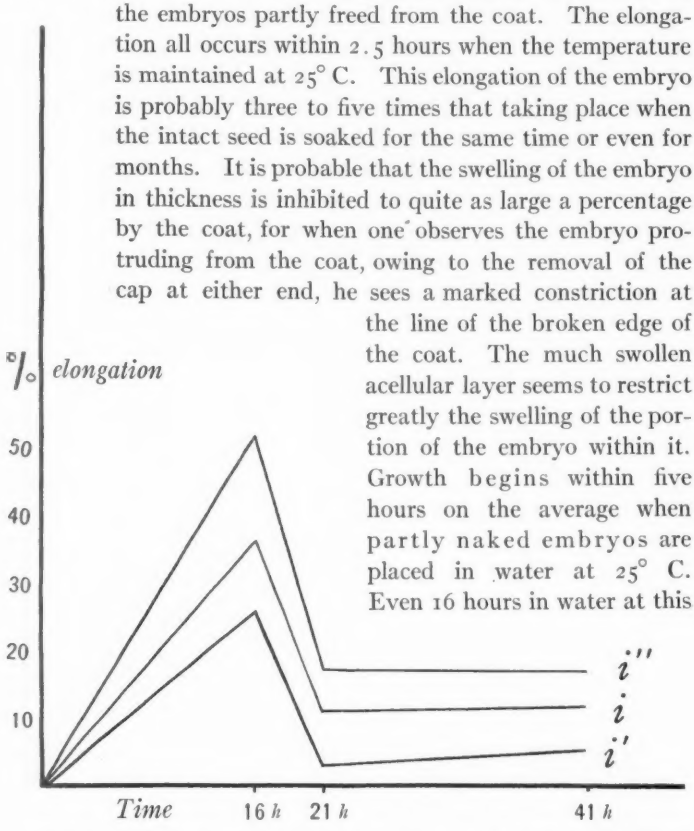

FIG. 7.—Percentage of elongation of 14 *Alisma* embryos with seed coats removed at both ends, soaked 16 hours in water, then placed in 2 mol. cane sugar solution for 25 hours: *i*, curve for the 14 embryos studied; *i'*, curve for the embryo showing the greatest swelling; i'', curve for the embryo showing least swelling.

temperature shows rather slight growth, although the average elongation is about 36 per cent. This all emphasizes the main rôle of osmosis and imbibition in the early very rapid elongation of

the embryo. The great elasticity of the embryo as compared with that of the intact seed is also noteworthy. The results of this section enable one to approximate the force (imbibitional osmotic) with which the embryo of *Alisma* in the saturated condition is pressing against the coat; this approximates 100 atmospheres (**24, 27**).

ATKINS (**34**) finds in certain legume seeds that only imbibitional forces are at work taking up water until growth actually begins; that is, a plasmolytic agent (KNO_3) does not inhibit the rate or final total amount of water taken up. In *Alisma* seeds, whether the coats are intact or not, as is seen from the experiments in this and previous sections, semipermeable membranes are functional from the beginning. With the coats broken, it is very likely protoplasmic membranes that are functional. It is evident that ATKINS' findings are not of universal application.

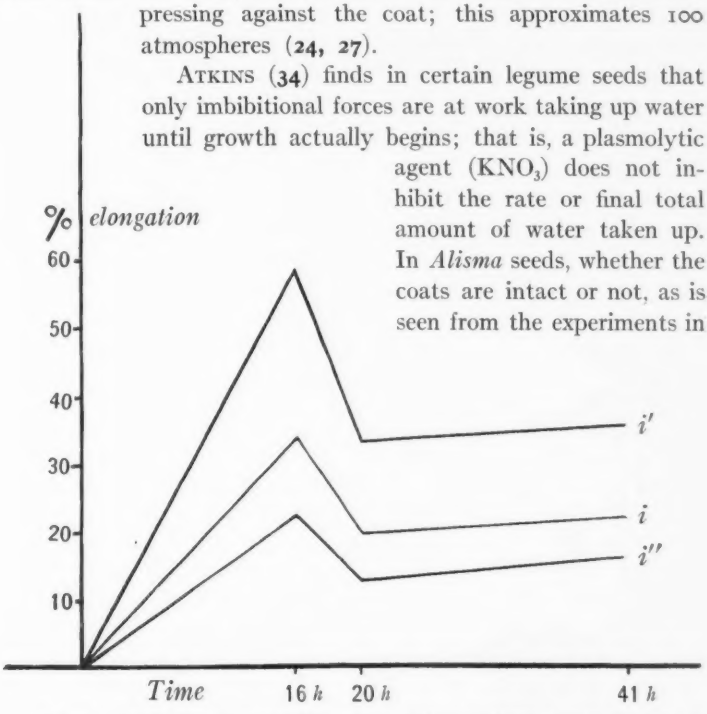

FIG. 8.—Percentage of elongation of 13 *Alisma* embryos (coats removed at both ends) placed in water for 16 hours, then transferred to mol. cane sugar solution for 25 hours: measurements made on dry embryos, after 16 hours in water, and after 5 and 25 hours in the sugar solution; *i*, curve for the 13 embryos studied; *i'*, curve for the individual showing most swelling; *i''*, curve for the individual showing least swelling.

5. ELASTICITY OF THE SEED COAT.—An attempt was made to determine the elasticity of the seed coat of *Alisma*. Thirteen good seeds freed from the ovary wall were selected and the length measured from the crown of the arch to the tip of the big arm and the width across the middle of the two arms. The seeds were soaked one day in water and remeasured and placed in 2 mol. cane sugar solution and measured after 15 and 24 hours. They were then transferred to water for one day and measured; then followed four days in dry air with the final measurement. Table XIII shows the modifications brought about in the two dimensions by the various treatments. The slight shrinkage caused by the transfer from water to cane sugar is especially noticeable. It shows the very slight elasticity of the seed coat as compared with the embryo. The total elongation of the intact seed, which is reached after two or three hours and becomes no greater even after months, is also slight (10 per cent) as compared with the embryo (19 per cent in 2.5 hours, 30.8 per cent in 5 hours, and 36.1 per cent in 16 hours).

TABLE XIII

Thirteen *Alisma* seeds, ovary wall removed, seed coats intact, soaked in water, followed by 2 volume mol. sugar solution, followed by water, and finally dried in air to get an estimate of the elasticity of the coats. Temperature 25° C.

TREATMENT OF THE SEEDS	WIDTH IN SCALE LENGTHS		LENGTH IN SCALE LENGTHS		PERCENTAGE OF INCREASE OVER DRY SIZE	
	Total	Average	Total	Average	In width	In length
Air dry	334.0	25.7	905.8	69.7	.....	.....
1 day in water	494.0	38.0	993.5	76.4	14.0	9.8
15 hours in sugar solution	481.0	37.0	963.0	74.1	11.0	6.3
24 " " " "	478.0	36.8	952.0	73.2	10.0	5.3
1 day " water	508.0	39.6	996.5	76.6	17.0	10.0
4 days " air	447.5	34.4	931.0	71.6	3.0	3.0

6. OXYGEN PRESSURE IN RELATION TO THE GROWTH OF ALISMA SEEDS AND SEEDLINGS.—We have already shown that the failure of *Alisma* embryos to break the coats, as well as their dormancy, is due to the fact that their possible imbibitional and osmotic swelling can be only in small part consummated in the intact seed. It has long been established (NOBBE, *Samenkunde*. 1876) that the swelling of seeds is merely a mechanical process which goes on

independently of oxygen supply. It is evident, therefore, that lack of oxygen cannot be the cause of dormancy in these seeds. MAZÉ (**20**) finds that seeds of most land plants will not germinate under water, and that storage under water in otherwise good germinative condition soon leads to loss of vitality. Good aeration of the water or addition of hydrogen peroxide to it will lead to germination under water. In contrast to this, GUPPY (**15**), FISCHER (**12**), and others have shown that seeds of many water plants may lie in water for years without germinating and still retain their vitality. This in itself suggests that the seeds of water plants are very different from land plants in their behavior toward a deficiency in oxygen. In *Alisma* we have shown that the embryo in the intact imbibed seed lacks much of being fully saturated with water. This probably holds for the seeds of many water plants, as one of us (**6**) has pointed out, and may in part account for the lack of injury from long storage under water. So far as investigated, seeds of water plants have extremely low minimum oxygen pressures for germination, if indeed any free oxygen is necessary for the process. The fact that their physiological processes can go on normally under water probably accounts in part for the capacity for water storage. One of the authors (**6**) has already pointed out this fact for seeds of several water plants.

During this investigation we have made a large number of experiments determining the relation of the amount of oxygen pressure to the growth rate, course of development, and chlorophyll formation in *Alisma* seeds and seedlings. We need mention only a few of the data and general results.

In all the experiments with the effect of various oxygen pressures on germination and development, the embryos used were partially freed from the coats and in an uninjured condition. Part of the experiments were run *in vacuo* and part by the displacement of air by hydrogen. In general, the cultures in hydrogen did not do as well as those *in vacuo*. This may be due to a slight toxicity of hydrogen itself or to some impurity in it. The hydrogen was derived from the electrolytic dissociation of water and freed from oxygen by passing it through a quartz tube filled with small copper disks and heated white hot with a blast flame. The hydrogen

proved to be essentially free from oxygen, as shown by its failure to color potassium pyrogallate. The experiments *in vacuo* were performed as follows. The seeds freed from coat cap and soaked in water at 0° C. for 12 hours were placed in 30 cc. distilling flask bearing about 20 cc. of distilled water. The main neck of the distilling flask was sealed off just above the side neck, and the side neck reduced to a capillary tube at one region. A Geryk air pump (capable of exhaustion to less than 0.1 mm.), protected by a drying system with great water-absorbing capacity, was attached to the side neck and the flask exhausted. The flask was then placed under water at 35° C. for one-half hour and the exhaustion continued. During the process the water generally boiled down to 5 or 10 cc., depending upon the bore of the capillary region. While still maintaining the boiling and exhaustion, the capillary was carefully sealed off.

Since toxicity entered into the results with hydrogen, we will report only experiments *in vacuo*, and of these only 2 of the 30 or more cultures need to be given. In these experiments 10 seeds were used in each culture. The controls were in Stender dishes bearing 0.5 cm. column of distilled water. The cultures were run in total darkness at a temperature of 21° C. for 21 days.

TABLE XIV

Length of the seedlings *in vacuo*		Length of the seedlings in full air pressure	
I	II	I	II
40 mm.	35 mm.	70 mm.	70 mm.
30	35	60	60
30	35	60	60
30	40	50	60
40	40	50	60
25	30	45	55
25	30	45	55
25	30	40	50
32	30	40	50
25	15	40	50
Av. length. . 30 mm.	32 mm.	50 mm.	57 mm.

The imbibed embryo in the intact seed is approximately 2.5 mm. long, so that the total elongation in the vacuum cultures equals

28–30 mm., 1120–1200 per cent; while in full air pressure, other conditions the same, an elongation of 48–55 mm., or 1820–2200 per cent, occurs. In these conditions only the stored food of the embryo is available for growth. It is evident that there is a much more economical use of the food in the presence of oxygen than in its absence, and still the growth is very considerable in its absence. It is possible that the use of nutrient solutions would have induced much greater growth, for it is certain that much leaching (**35**) of the salts of the embryos must have occurred in these experiments.

Even in light, no leaf branches are ever formed in the seedling grown *in vacuo*, nor does any chlorophyll develop. In weak diffuse light at 25° C., the seedling did not develop chlorophyll even after a month, if the air pressure was less than 5 mm. If the air pressure exceeded 5 mm., chlorophyll generally developed in these conditions. This shows that the oxygen pressure demanded for greening is rather slight though easily measurable. Seedlings in darkness under full air pressure all develop one or more leaves. If the air pressure is reduced much below 5 cm., no leaf branches develop even after two months. If the air pressure is more than 15 cm., there is an abundant formation of leaf branches. The development of a primary root also seems to be dependent upon oxygen pressure. Nearly every seedling growing in a drop culture in a Van Tieghem cell shows the early development of a primary root, as do seedlings growing on the water surface. Fewer seedlings developing at the bottom of a considerable layer of water show primary roots, and none in cultures with air pressure reduced much below 5 cm. The growth of water seeds and seedlings *in vacuo* stands in contrast to the facts found by SHULL (**30**), BECKER (**2**), and ATWOOD (**1**) for the seeds of land plants, where definite oxygen minima exist.

IV. General discussion

Work to date has shown that delayed germination of seeds is secured in a variety of ways: by almost absolute exclusion of water by seed coats, as in the "hard-seeded" legumes and species of several other families; by limiting the degree of swelling of the embryo by surrounding structures, as in *Alisma* and probably

in seeds of a large number of water plants; by reduction of oxygen supply below the minimum necessary for germination by means of seed or fruit coats or other structures, as is the case in *Xanthium* (30) and a number of other Compositae (2), in *Chloris ciliata* (14) and other grasses, and in *Datura Stramonium;* and finally, perhaps, by deficiency in salts. To these must be added delays due to embryo characters, as slowness of acid development in the hypocotyl of the haw, apple, peach, and perhaps other Rosaceae. The investigation of the mechanics of delayed germination in any particular seed should consider all of these possibilities, and not assume, under the incubus of the overworked stimulus conception with its indefiniteness and implied inscrutability, that the cause must lie in the embryo. The German investigators, with few exceptions, have been especially prone to the stimulus view and its implication that in characters of the embryo alone dormancy is determined. They of course except the seeds that are known as "hard-coated." This inclination to the stimulus conception is evident in FISCHER'S (12) interpretation of his results with seeds of water plants. We find BEHRENS (3) assuming that the pricking of intact seeds has its effect only through stimulus action, and not implying at all the possibility of greater oxygen admission or mechanical weakening of the coats. The same can be said of KIESSLING (17) working with various grasses, and KINZEL (18) with the "light-hardened" seeds of *Nigella*.

Again, in their work with light-sensitive seeds, with the single exception of GASSNER (14) on *Chloris ciliata*, the German investigators have assumed that the light has its effect always upon the embryo and never upon the coat. One must remember that the coat is most strongly exposed, and that it reduces the light intensity greatly before it reaches the embryo, at least in heavy coated seeds like *Phacelia tanacetifolia*. It is quite as possible that photochemical changes are occurring in the coat as in the embryo. NEUBERG has found that about every group of organic substances, polysaccharides, fats, proteins, etc., is hydrolized, and often the completely hydrolized products are further transformed by light, if iron, manganese, or certain other salts are present. These decompositions occur in non-living substances, so there is no reason for

assuming that photochemical transformations are limited to the embryo and are not occurring in the more highly illuminated surrounding structures. Again, it is well established that light increases the permeability of protoplasmic membranes to various solutes. The possibility appears that it may likewise increase the permeability of non-living semipermeable membranes of the coat to oxygen or perhaps other materials needed for germination. Likewise, they have failed to see that the fixation of the carbon dioxide released in respiration may in some cases supply the oxygen needed for germination. It is strange that these workers have never done the evident thing of experimenting with naked embryos to see what their needs are in the way of germinative conditions. In seeds like *Viscum album* it is evident that the demand for light cannot be related to the seed coats, but in most light-sensitive seeds the conclusion is not evident.

It is established, however, that not all cases of delayed germination are due to coat characters acting in conjunction with embryo characters, but there are some embryos, or rather organs of some embryos, that are dormant when entirely naked. But even here it is probable that the explanation of the action of forcing agents will not need to be veiled under such an indefinite term as stimulus. ECKERSON (10) has shown that the hypocotyls of haw and apple seeds are dormant because of slowness in developing acidity, a condition necessary for proper water absorption for growth and for the formation and action of digestive and perhaps respiratory enzymes. The acid development begins even during swelling in all organs of most seeds, as it does in the cotyledons of the haw and apple, but the hypocotyls in the latter seeds remain slightly basic or neutral for a long period. It has been shown (9, 10) that temperature and water and oxygen supply determine to a great degree the speed of acid development in the hypocotyls mentioned. Likewise, the seed coats and carpel walls play an important rôle here, so far as they affect these factors. Acids are good forcing agents for dormant hypocotyls; apparently, they have their significance in this fundamental relation to water absorption and to the formation and activity of enzymes. LEHMANN (19) has suggested that light and agents and conditions that substitute for

light in light-demanding seeds have their effect in bringing about hydrolysis of proteins. His evidence is hardly conclusive. It is quite possible, however, that dormancy in an embryo may be due to the abeyance of some single simple process under a given set of conditions. If so, it may be any one of several aside from protein hydrolysis. If such cases exist, they stand more or less in contrast with the case worked out by ECKERSON (10).

FISCHER found phosphoric and certain organic acids more effective as forcing agents for *Alisma* than their dissociation would insure on the basis of hydrogen ions. This we are unable to explain. Of course FISCHER worked with chaffy fruit coats intact, and it is possible that organic acid had its main function in acting as a culture medium in the carpel wall, so that fungi and bacteria could get a hold on the seed coats. The seed coats seem to form excellent media for the growth of fungi and bacteria. Both layers of cells are very rich in reducing substances. If cultures were started in the carpel wall, they would probably thrive on the seed coats. In contrast to the seed coats, the embryos are very resistant to the attack of fungi and bacteria. As FISCHER points out, one gets good germination in putrefying cultures. This we are quite confident is not due to acid formation, but is rather due to an organism actually decomposing the coats. At 25° C., 0.001 N hydrochloric acid will not cause the rupture of the coats in *Alisma* even after many days' soaking, and this concentration of acid permits but little growth in the naked embryo. It is evident then that the acid could not be strong enough to cause the swelling of the embryo to break the coat and still allow the embryo to grow after it had broken the coat. FISCHER had the preconceived notion that the embryo was dormant, and therefore neglected the possibilities that have proved to be much more to the point. This makes it probable that in putrefying waters in nature the germination is brought about by the direct attack of the seed coats by the organisms, and not through the action of acids formed by the organisms. In the face of the fact that he could soak *Sagittaria* seeds in 1 mol. copper sulphate for 5 days and later secure their germination by treating with acids and placing in water, FISCHER comes to the conclusion that the seed coats are very permeable to solutes in general.

This has been a standing assumption for a long time, but an assumption not backed by evidence. The claim has received lately most telling counter-evidence by the work of BROWN (**5**), SCHRÖDER (**28**), and SHULL (**29**), from which it appears that semipermeable membranes are of rather common occurrence in seed coats. We have already given evidence for a sodium chloride excluding membrane in the coat of *Alisma*. We find likewise that while the intact seed is not injured by many days' soaking in 1 mol. copper sulphate, the naked embryo will not grow markedly in a 0.001 mol. solution of the same salt.

The conclusions drawn in this paper must be applied only to *Alisma* seeds, but it will be surprising if they do not hold for the very similar seeds of *Sagittaria*, and for the less similar seed of *Eichhornia*, and perhaps for the seeds of many water plants.

The power of acids to hasten the germination of the haw must not be identified with the forcing effects in *Alisma*. In the first case, the effect is on the dormant hypocotyl itself, and in the second, on the coat in the main. In the first case it is hardly necessary to confuse the matter by calling the effect a stimulus, and in the second such an explanation is quite aside from the point.

The peculiar effect of copper sulphate solutions on the germination of acid-treated intact seeds shows the danger of assuming that such effects of salts are on the embryos rather than the coats when intact seeds are used.

We wish to express our gratitude to Dr. SOPHIA ECKERSON for most of the microchemical determinations reported in this paper.

V. Summary

1. Dormancy in the akenes of *Alisma Plantago* is due to the mechanical restraint of the seed coat. This restraint enables the seed to be in water for years without germination.

2. The chaffy carpel wall plays no part in the dormancy. Of the three layers of the seed coat (the outer single layer of reddish-brown cells, the inner single layer of white cells, and the lining acellular pectic hemicellulose material), the outer seems to play no

part in the delay. The effect must be attributed to one or both of the inner layers.

3. As the intact fruit lies in water in the saturated condition, the embryo itself does not half (probably not more than one-fourth or one-fifth) consummate its possible imbibitional and osmotic swelling. The embryo only partly swollen thus lies for years in water, restrained in its swelling by the seed coat, against which it must be exerting a pressure of approximately 100 atmospheres.

4. The air-dry seed (freed from the carpel wall) when placed in water swells rapidly. It increases 40 per cent of its air-dry weight in the course of two hours. From this time on it shows a slow increase to 50 per cent of its air-dry weight, which is maintained constant even after long periods of soaking. A large part of the water absorption is due to the hydrophilous pectic and hemicellulose substances of the seed coat, especially the inner acellular layer.

5. When the coat cap is removed from the large end of the embryo, thus leaving the embryo more free to continue its imbibitional and osmotic swelling, the seed swells even much more rapidly, reaching about 60 per cent of its air-dry weight in two hours and more than 100 per cent after 20 hours. The imbibitional and osmotic swelling gradually passes into growth enlargement.

6. With the coat cap removed from both ends of the embryo and the seed placed in water, the embryo elongates 19 per cent of its air-dry length in 2.5 hours. This would extend the embryo at least 20 per cent the length of the swollen seed beyond the limits of the seed coats. This elongation is all imbibitional and osmotic, involving no growth. Five hours' soaking gives an elongation of 30 per cent of the air-dry length of the embryo. This is only in very small part due to growth. Sixteen hours' soaking gives 36 per cent elongation, which involves considerable growth. Imbibitional and osmotic swelling alone would extend the embryo of *Alisma* far beyond the limits of the swollen seeds.

7. The seed coat is composed almost entirely of pectic substances which are very easily transformed by weak acids and bases. Besides bringing about chemical changes in these substances, acids and bases change their water relations as is true of hydrophilous colloids in general.

8. There is some evidence that acid increases the imbibitional force of the embryo. If such is the case, the increase is very slight. Bases increase greatly the rate of elongation of embryo.

9. It seems that the effect of acids and bases on the germination of *Alisma* seeds is largely to be explained by a weakening of the seed coats, so that the imbibitional and osmotic swelling of the embryo is capable of breaking away the coat cap at the large end of the embryo. It is possible that they are also in part effective by increasing the force of the imbibitional and osmotic swelling.

10. This gives a chemical-physical explanation which displaces the vague implications of the term "stimulus."

11. The seeds of *Alisma*, as of the seeds of water plants in general, are capable of lying in water for years in the imbibed condition without losing their vitality. In contrast to this, seeds of land plants will withstand such storage for a relatively short time.

12. The embryo of *Alisma*, at the expense of its stored foods alone, is capable of more than 120 per cent elongation in total absence of oxygen. For various other phases of its development (greening, branching, development of primary root) it requires some free oxygen. The greening requires at least 5 mm. of air pressure, the branching more than 5 cm. of air pressure, and the general development of primary roots still more.

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SPORE CONDITIONS IN HYBRIDS AND THE MUTATION HYPOTHESIS OF DE VRIES[1]

EDWARD C. JEFFREY

(WITH PLATES XXII–XXV)

At the end of the sixteenth century CHARLES DE L'ECLUSE became professor of botany in the University of Leyden. He brought with him from Vienna to his new abode plants of the tulip, which had been introduced from Turkey into southeastern Europe. These formal plants he cultivated with great success in his own garden, and was fortunate in arousing a keen and even commercial interest among the phlegmatic Dutch; so much so that when the excitement was at its height, his garden was invaded and many of his choicest varieties stolen. Thus arose the famous "Tulipomania," which has been immortalized by DUMAS. Three hundred years later another Dutch botanist has become the author of another botanical sensation, which will doubtless also live in history. Professor DE VRIES has initiated the investigations on the genus *Oenothera* in connection with the hypothesis of mutation or saltatory origin of species. His discovery first that *Oenothera Lamarckiana* and subsequently that other species of the genus are capable of producing distinct so-called elementary species when cultivated on a large scale has very properly attracted a great deal of attention, particularly in the continent of North America, which prides itself, not altogether without reason, on its openness to new ideas. Thus it has come about that the genus *Oenothera*, and in particular *O. Lamarckiana*, has become the battleground of the mutation hypothesis on the botanical side. We have had during the past decade a veritable "Oenotheromania" comparable to the "Tulipomania" of three centuries ago.

There have been many objections raised to the mutation hypothesis of DE VRIES, particularly on the part of geneticists of

[1] Contributions from the Phanerogamic Laboratories of Harvard University, no. 70.

standing. The Englishman BATESON was apparently the first of these to record the opinion that *Oenothera Lamarckiana* was a hybrid, and he has been followed in this expression of opinion by many American scientific students of plant breeding. The greatest difficulties which have arisen in connection with *Oenothera Lamarckiana*, as studied by geneticists, have been in connection with its origin, and all herbaria, which could be supposed in any way to throw light on its original much discussed appearance, have been ransacked, without apparently a final or decisive result. The antecedents of this much disputed species, however, are now of less importance in view of the fact that other and possibly all species of *Oenothera* are likewise "mutating." The question of mutation, so far as it depends for its resolution on the study of *Oenothera*, has fortunately at the moment a much wider basis than the conditions found in any individual species of the genus, concerning the origin of which there may be serious doubt. As will be shown later in the present article, many of the species of *Oenothera*, if not all, are in the same peculiar and obviously extremely significant state as is *Oenothera Lamarckiana*. Attempts have been made to synthesize *O. Lamarckiana* from other American species, but this line of attack seems to lose some of its importance from the fact that almost any wild American *Oenothera* has apparently the same genetical peculiarities and the same power of "mutating" as has *Oenothera Lamarckiana* itself.

It has long been recognized on the part of scientific students of plant breeding that spore infertility is an important characteristic of hybrids. This view, originally expressed well on to a century ago, has lost none of its force in the intervening years. It is further recognized quite generally that relatively fixed hybrids may be produced. The opinion is at the present moment rapidly gaining ground, both on the zoological and on the botanical sides, that many of our wild species of plants and animals are in reality more or less fixed hybrids. In the case of plants we have peculiar advantages in connection with the recognition of this fact. We owe the most important results which have yet been produced on this continent, in this direction, to the studies of BRAINERD upon spontaneous hybrids of the violets and Rosaceae, for the most part

published in *Rhodora*. BRAINERD'S line of attack has been from the systematic, and, to a less extent, from the genetical side. It is the purpose of the present and succeeding articles to demonstrate evidence from the morphological side that hybridism is extremely widespread in nature, among the higher or angiospermous plants in particular, and that there is every reason to suppose that it has been an agency of great importance in *multiplying* species, although it is logically inconceivable in the present state of our biological knowledge that it could have presided at their *origin*.

In the following paragraphs the general conditions of sporogeny, so far as they are related to the matters under consideration, will be discussed and compared in the main groups of the embryophytes.

Beginning with the liverworts, the writer has examined a number of examples from the Marchantiales, Anthocerotales, and Jungermanniales (both acrogynous and anacrogynous), with the result that in no case which has come under his observation are there present any abnormal products in connection with spore-formation, unless the elaters can be considered abnormal structures in this respect. These elements, although undoubtedly derived from potential spore mother cells, are perfectly normal and have no significance in relation to a possible genetical impurity, any more than have the abortive spores and spore mothers found in the case of heterospory. In *Boschia*, as is shown in LEITGEB'S classic monograph, the elaters are represented by sterile cells without tracheary thickenings. In *Sphaerocarpus* the multinucleate condition of the elateriform cells clearly reveals their morphological derivation from spore mother cells. Fig. 1 illustrates the condition of the spores in *Marchantia polymorpha*, as an example of the liverworts. The elaters and spores are clearly seen, the former as spirally thickened bands, and the latter as dark or lighter spherical bodies. Where the spores are light in hue, the plane of section does not include the protoplasmic contents. Whether light or dark, the spores are of equal size and unshrunken.

An examination has been made of the sporogonia of a considerable number of the leafy mosses, with results, as far as they have gone, similar to those presented by the liverworts. The only genus examined which showed imperfect or abortive spores was

Sphagnum. As is well known, this genus is extremely variable, and the species are often difficult to distinguish. It appears accordingly not improbable that the abortion of spores sometimes found here may be correlated with hybridism in nature. Fig. 2 shows the homogeneous character of the spores in the common species *Polytrichum commune*. As is the case in the leafy mosses or Phyllobrya in general, there are no elaters present.

Numerous representatives of the Lycopodiales, heterosporous and homosporous, were examined and abortive spores were seen to be characteristically absent. Five species of *Lycopodium*, as well as *Phylloglossum*, *Psilotum*, *Tmesipteris*, *Selaginella*, *Isoetes*, *Lepidodendron*, *Spencerites*, etc., all yielded uniform results.

One species of *Equisetum* has long been recognized to be of hybrid origin, namely *E. littorale*, which from its anatomical and external features is a hybrid between *E. arvense* and *E. limosum*. It is one of the few conventionally recognized hybrid species, and is characterized by the large number of abortive spores which are found in its sporangia. Miss HOLDEN has recently published in *Science* an interesting account of abortive sporogeny, accompanied by blended anatomical features, in *Equisetum variegatum* var. *Jesupi*. It appears beyond question, from Miss HOLDEN'S work, that this variety is the result of a cross between *E. hiemale* and *E. variegatum*. It is further probable from her work that other recognized varieties of species of *Equisetum* are likewise hybrids in their origin. Fig. 3 represents a section through part of a sporangium in *E. hiemale*. Although all of the spores are not equally in the plane of section, it is clear that they are normal and unmingled with abortive individuals. In fig. 4 is shown a view of a section through a sporangium of *E. variegatum* var. *Jesupi*. Here there are practically no sound spores, unless one to the right of the figure can be considered sound. Even the spores of relatively large size and dark (protoplasmic) contents are abnormal. The smaller and abortive spores, which enormously predominate in the figure, have very scanty protoplasmic contents or none at all. Contrary to statements usually made in regard to abortive spores of *Equisetum*, the elaters are present, although not normally developed. They can be seen as circles bounding the spore proper

at an interval. They do not ordinarily separate into the usual bands, which is doubtless the reason their presence has not been heretofore recognized in the case of abortive *Equisetum* spores. Examination has been made of a number of sporangia of *Calamites*, and here, as in the living *Equisetum*, when uncontaminated by species crossing, the spores are perfect, although without the elaters, which appear likewise to have been absent in the mesozoic *Neocalamites*.

The ferns may next be considered, and for the purpose of the present investigation they may be divided into the Polypodiaceae and the remaining ferns. Taking the latter first, no evidence of hybridization in the recognized older groups of ferns has been found in this connection. Fig. 5 illustrates in section a sporangium of *Angiopteris evecta*. The spores are obviously all perfect. Examination of the genus *Marattia* has led to similar results. Other representatives of the Marattiaceae were not available. The Ophioglossaceae, as represented by the three genera *Ophioglossum*, *Botrychium*, and *Helminthostachys*, were studied and no evidence of abortive spores found in the sporangia. Representatives of the Gleicheniaceae and Schizaeaceae yielded similar results. *Osmunda regalis*, *O. cinnamomea*, and *O. Claytoniana* have strikingly perfect spores. *Todea barbara* showed similar conditions. Hymenophyllaceae, as represented by *Hymenophyllum* and *Trichomanes*, and the Cyathaceae by *Alsophila* and *Dicksonia*, yielded the same results. Of the heterosporous ferns, *Marsilia* and *Pilularia* were available, and these showed uniform spores. *Azolla* of the Salviniaceae has uniform spores.

Numerous cases of hybridism among the Polypodiaceae, accompanied by greater or less spore abortion, are known. The writer has personally examined only one hybrid fern of this group, namely the so-called *Adiantum hybridum* of gardeners. The spores here are apparently completely abortive, at any rate in greenhouse specimens. It will be unnecessary to enlarge upon the hybridism of the Polypodiaceae further, because recently an admirable statement has been published by BENEDICT,[2] in which he discusses the subject fully. He reports 11 natural hybrids for the eastern

[2] BENEDICT, R. C., Bull. Torr. Bot. Club **36**: 1909.

states, representing three genera. The status of some of these hybrids is beyond question because the cross has been repeated experimentally. Spore sterility is a marked feature in such cases. A large number of examinations of normal species has been made in the Polypodiaceae in the present connection, and uniformity of spore-formation has been found in such cases.

The Cycadales are of considerable interest from the evolutionary standpoint. The writer has been unable to find evidence of imperfection in the mature output of the sporangium. Two species of *Zamia*, as well as *Cycas revoluta*, *Stangeria paradoxa*, *Bowenia* sp., and *Microcycas*, were all examined with unvarying results. Fig. 6 shows part of a section through the microsporangium of *Zamia floridana*. The spores appear round where seen in face view, and somewhat crescentic when examined in profile. Both views may be seen in the illustration.

The monotypic *Ginkgo*, the sole survivor of a group once abundant in the Northern Hemisphere, has pollen which, in the large number of cases in which I have examined it, has proved to be singularly free from variation in size in the same sporangium. I have yet to see an imperfect microspore of *Ginkgo*. Fig. 7 shows the pollen as seen in a transverse section of the microsporangium. It will be noticed that the pollen grains present different appearances according to the plane of section and to the angle of incidence. The fact that the pollen in this interesting genus is winged as in *Pinus* can already be seen with the magnification employed in fig. 7. Fig. 8 presents a much more enlarged view, and the wings can be clearly discerned. It is curious that this striking feature of the pollen appears to have escaped notice. The wings bear the same relation to the cells of the microgametophyte within the pollen as they do in the Abietineae, and the number of prothallial cells in *Ginkgo* corresponds with that found in the Abietineae and not with the conditions found in the cycads, with which they are usually more nearly associated. A contribution from this laboratory dealing with the extremely striking features of resemblance between the Ginkgoales and the Abietineae will appear very shortly. The resemblance extends to nearly all the more important details of the reproductive and vegetative organs of the two groups.

The writer has had the opportunity of examining large quantities of coniferous material of all the subtribes, since his own studies have been particularly in this group. The Abietineae, Araucarineae, Podocarpineae, Taxineae, and Cupressineae in the broad sense, have all been studied in most of their genera, and in the case of the larger genera in a number of species. Perfection of pollen development was found to be characteristic of the microsporangia of this important group. The only case of pollen abortion which has come to light during the present investigation is that furnished by one species of *Abies*, to be described later. Fig. 9 illustrates the pollen conditions in *Araucaria Cooki*, cultivated in a greenhouse. It will be seen that the grains are all perfect, the difference of size being due to their greater or less inclusion in the plane of section. The greenhouse conditions have obviously not affected the morphological perfection of the pollen. Fig. 10 shows part of the same section under a higher degree of magnification. The numerous prothallial cells, which are a feature of the organization of the gametophyte produced in the pollen grains of the Araucarineae, can be seen.

The Gnetales have been studied in all their three surviving genera. *Welwitschia* has entirely perfect grains. The same statement holds for the three or four species of *Gnetum* which I have examined in this connection. Two species of *Ephedra* were examined, with the result that the pollen appeared to be without tendency to abortion. It is perhaps of interest to record, in this connection, that the pollen of *Ephedra* and *Welwitschia* are strikingly alike externally, both being characterized by spiral striation of the external coat of the oval grains. This feature was not found in the case of *Gnetum*. It has not been thought necessary to introduce an illustration of the microspores of the Gnetales, although several were prepared for this purpose.

The Monocotyledons may next be considered. Fig. 11 shows the pollen conditions in a wild species, *Iris versicolor*. The pollen is obviously perfect in development. Many hybrids of the various species of *Iris* are grown in gardens, in fact most of the more attractive cultivated irises are the result of hybridization. Fig. 12 illustrates the pollen conditions in a hybrid improvement

of *Iris germanica*. The species of *Iris* are usually quite free from any indication of hybrid contamination, with the possible exception of *Iris Hookeri*, which has not been fully studied. In fig. 12, the pollen is largely abortive and is reduced in amount, as is frequently the case in hybrids of all kinds.

Fig. 13 shows the pollen conditions in *Lilium canadense*. The grains are here quite perfect. Similar observations were made on *Lilium philadelphicum* and the common Easter lily. Fig. 14 illustrates the pollen abortion which is the usual condition in our cultivated *Narcissus*, nearly all of which are known to be of hybrid origin. The shriveled and abortive condition of a large number of the grains is quite evident. Fig. 15 pictures the condition of the pollen in *Lachenalia Nelsoni*, a plant very frequently grown in greenhouses in winter. This hybrid between two natural South African species of the genus was made by an English clergyman, in window culture, many years ago. Most of our garden tulips show their hybrid origin by large quantities of abortive pollen. A very large number of observations has been made upon monocotyledonous cultivated hybrids, with the unvarying result that hybridism was characteristically connected with a considerable amount of pollen degeneracy. On the other hand, monocotyledons grown in the greenhouse under obviously somewhat unnatural conditions, where of pure specific origin, showed, in all cases examined, good pollen. This was the case, for example, in species of *Gasteria*, *Agave*, etc.

We now advantageously turn our attention to the very large natural group of Dicotyledons. Here hybridization both natural and resulting from the intervention of man is extremely common. We may first consider with advantage the Rosaceae, in view of BRAINERD'S very interesting studies on this family. This investigator has shown that a great many natural hybrids of *Rosa* and *Rubus* occur in nature. The morphological observations made in this family entirely confirm BRAINERD'S results, and extend them in an apparently very interesting way, which will be summarized here, a fuller account being reserved for future publication. Not only are certain of the Rosaceae recognizable as hybrids, on account of their transitional external features of organization, Mendelian

phenomena, etc., but certain others, which have not revealed themselves as hybrids in these ways, are clearly such, as a result of the study of their spores. As an example let us take our mountain ashes (*Rosa* or *Rubus* furnish just as good illustrations, or in fact almost any of the larger genera of the Rosaceae in the Northern Hemisphere). In Europe and Asia it is generally recognized that the valid species of *Sorbus* have between them a number of natural hybrids. In the eastern and northern region of North America, we have two common species of mountain ash, *Sorbus americana* and *Sorbus sambucifolia*. These have been shown in connection with the present investigations to have pollen which is to a considerable degree abortive. The same observation has been made in regard to the European *Sorbus aucuparia*. In fact, the only species of *Sorbus* yet examined in this connection with perfect pollen is one growing in Japan. Or turning to another genus, *Prunus*, most of the species examined had imperfect pollen. Of the common species in New England investigated, the only one with perfect pollen was *Prunus serotina*, which flowers somewhat later than the others and is consequently phenologically isolated, just as is the case geographically with the Japanese *Sorbus* mentioned above. Illustrations of a similar nature could be given to an almost unlimited extent from studies on the Rosaceae, which are as yet far from complete. We have consequently to distinguish in the case of the Rosaceae, if the morphological features are taken into account as well as the data of systematic botany, three kinds of individuals, namely pure species, recognized species which from the condition of their pollen are in reality concealed hybrids or crypthybrids, and recognized hybrids. In some genera the multiplication of species through hybridization is very large; for example, over 4000 species of *Rubus* have been described in Europe, and in this country SARGENT and others have described a very large number of species of *Crataegus*.

Nearly all our common garden herbaceous dicotyledons, which from the nature of things are capable of hybridization, are hybridized. For example, we may take the carnations, the petunias, the phloxes, the chrysanthemums, the calceolarias, certain primroses, etc. Without concerning ourselves further with the dicotyledon-

ous cases of hybridization, we may now advantageously turn our attention to the consideration of the Onagraceae, the family which has supplied the famous case of *Oenothera Lamarckiana*. We may conveniently begin with our common garden fuchsias, which are known to be of hybrid derivation from *Fuchsia magellanica*, an inhabitant of southern South America. Fig. 19 shows the interesting pollen conditions found in certain cases in the cultivated *Fuchsia*. Many of the grains are shriveled and empty. In other instances still less pollen perfection was observed; in fact in some flowers there is practically no functional pollen and the anther sacs do not open, but dry up without dehiscence. In still other cases the pollen does not definitely shrivel up, but the grains are of very unequal size and contain a very scanty supply of protoplasm.

Let us now turn to the genus *Epilobium*. Our common fireweed, which inhabits somewhat generally the Northern Hemisphere, is illustrated in fig. 16. The figure includes all four anthers, which are laid open in the plane of section. It will be observed that the pollen in their cavities is perfect. The illustration is purposely made to show a large amount of pollen to demonstrate how normal the pollen development is in *Epilobium angustifolium*, the common willow herb, or fireweed. Fig. 17 shows a similar section of the flower of *Epilobium hirsutum*, the giant willow herb, which occurs near ballast heaps and is not uncommonly cultivated in New England. Even with the low magnification used, it becomes at once clear that the pollen development is abnormal, some of the grains being smaller and without protoplasmic contents. Fig. 18 shows a higher magnification of two of the anther cavities in this species, and on the left may be seen two sound grains and five abortive ones. On the right only abortive grains appear. The pollen in certain species of *Epilobium* occurs in tetrads, and in a smaller number of others it is quite separate at maturity. Those with the latter condition of the microspores are quite commonly put under the genus *Chamaenerion*. *Epilobium hirsutum* consequently shows evidence of hybridism in the presence of abortive spores in its anther cavities. For comparison with this type, the pollen development of the genus *Rhododendron* in the Ericaceae was examined. In a pure species, such as *Rhododendron catawbiense*,

all the grains are alike and all the tetrads are similar to one another. There are a number of hybrid derivatives of this species in cultivation. Two of these, named *R. Metternichi* and *R.* Mrs. H. W. Sargent, have been examined in this connection. The conditions found are exactly like those presented by *Epilobium* in the stricter sense. Some of the tetrads of pollen are entirely abortive, some only partially so, and in still others all the grains are perfectly developed. An extremely interesting fact in the present connection is the statement in standard European works on the genus *Epilobium* that its species all hybridize naturally with one another. In our specimen of *Epilobium hirsutum*, we obviously have to do with one of these hybrid conditions.

We are now, without further consideration of the Onagraceae, which would take us beyond the scope of the present article, in a position to consider advantageously the genus *Oenothera*.' In fig. 20 is shown a portion of the anther contents of *Oenothera Lamarckiana*. Obviously the pollen is largely abortive. In fact, in his *Mutationstheorie*, DE VRIES does not conceal the fact that about one-third of the pollen of this species is shriveled and non-functional. Not only is this true of the species, but it is likewise true of its so-called mutants, some of the weaker of these, such as *O. nanella* and *O. lata*, having almost no good pollen. But it is not in *O. Lamarckiana* alone that one finds imperfect pollen. All of the numerous species of the genus which I have examined in this connection show more or less the same state of affairs. Fig. 21 illustrates the conditions in one of our commonest oenotheras, namely *O. biennis*. Even with the low magnification used, it is easy to see that the contents of the eight anthers shown photographically are far from normal. Fig. 22 shows the conditions as they appear under a higher magnification. Obviously here, as in *O. Lamarckiana*, a large amount of the pollen is sterile. All that can be said is that the genus *Oenothera* is in general, judging from its morphological condition, in a state of high genetical impurity. Fig. 23 shows a rather low magnification of several of the anthers of *O. Lamarckiana*. *O. grandiflora* is illustrated under the same degree of magnification in fig. 24. In this species the number of abortive pollen grains present is very small, and it is

in fact the least characterized by shriveled microspores of any species which I have yet had the opportunity to examine. It would seem consequently that it has either largely recovered from the effects of previous hybridization or else that the hybridizing ancestors have been less incompatible than those of, for example, *O. biennis*.

It is obvious that in the Onagraceae we have a peculiar condition, which is likewise present in other families of the angiosperms. Taking the Rosaceae as an illustration, there are in the various genera of this family numerous forms which are generally recognized on external characters as hybrids. Some of these conditions have recently been described by BRAINERD. In addition to admitted hybrids in the Rosaceae, there are a number of forms which very generally are admitted as good species on account of their relative constancy and the absence of observed intergrading types. In many of these recognized species, however, the morphological conditions, found in connection with the formation of the reproductive elements, are clearly those of hybrids. In view of the common occurrence of admitted hybridism in the family, these may properly be regarded as concealed or crypthybrids. The value of internal structure in the case of hybrids, as a key to the conditions involved, has not been sufficiently regarded in the past. It is obviously of the greatest importance in the Rosaceae. A further group of forms is found in the Rosaceae, where the reproductive structures are those of normal species elsewhere. That is to say, the pollen, the more readily investigated of the reproductive elements, is quite sound. This condition, for example, is characteristic of monotypic genera or genera with few well marked species (for example, *Kerria*, *Rhodotypus*, *Cydonia*, etc.). In genera with numerous species, contamination is very likely to be present, except in cases where the species are very distinct, or are isolated phenologically or geographically. For example, *Rosa* or *Sorbus*, geographically isolated in a mountainous or insular habitat, is genetically pure; while *Rosa* or *Sorbus* of continental or lowland origin is very likely to be genetically impure.

The principles cited above in the case of the Rosaceae have an obvious bearing on the conditions found in the Onagraceae.

Here, as in the larger family Rosaceae, we have the occurrence of admitted hybrids in certain genera (*Epilobium* and *Fuchsia*), side by side with recognized species. In other cases the recognized species have for the most part the same pollen conditions as those characteristic of hybrids. This state of affairs is found to a very large and striking degree in the genus *Oenothera*. Since the species of *Oenothera* to a very considerable extent, particularly those which have been used in investigations of a mutational or genetical nature (*O. Lamarckiana*, *O. biennis*, etc.), are under the strongest possible suspicion of hybrid origin, it follows that any conclusions drawn from their genetical behavior, in connection with the vexed problem of the origin of species, must be subject to a large degree of reserve. So far as the conduct of *O. Lamarckiana* and other species of the genus throws any light at all upon the species problem, it is in connection with the *multiplication* of species by hybridization, a very different matter indeed from the actual *origin* of species.

The relation of hybridization to "mutation" has recently been strongly emphasized for animals by GEROULD.[3] The reader is referred to this highly interesting and important contribution on the zoological side, for an expression of views very similar to the conclusions reached in the present article in the case of plants. In plants we have the great advantage of being able to a very large extent to supplement genetical and taxonomic data by the examination of internal organization.

The writer[4] has previously made a general statement on the questions here involved. GATES[5] has recently published an article in which he attempts to show that mutation and hybridization are independent phenomena. His conclusions seem to be fatally invalidated by the fact that his experiments were carried on upon *Oenothera* species and "mutants." The situation is apparently the same as would present itself if a chemist worked with impure chemicals or a physicist with mixed radiations.

[3] Species-making by hybridization and mutation. Amer. Nat. **48**: no. 570.

[4] The mutation myth. Science N.S. **39**:488–491. 1914.

[5] Breeding experiments which show that hybridization and mutation are independent phenomena. Zeitschr. Induktive Abst. und Vererb. **2**: Heft 4.

Summary

1. Spontaneous hybridization is comparatively rare among lower plants, but very common in the angiosperms.

2. A long recognized criterion of hybridism is sterility, partial or complete, of the reproductive cells. In plants this is recognized with particular ease in the case of the pollen.

3. In forms which are ordinarily recognized taxonomically as species, pollen infertility frequently indicates past genetical contamination.

4. In families such as the Rosaceae and the Onagraceae, we find grading into each other recognized species and recognized hybrids, having in common the character of partial or complete reproductive sterility, most easily recognized in the organization of the pollen.

5. This situation points inevitably to the hybrid origin of these abnormal species.

6. Species of this type may conveniently be called crypthybrids.

7. The species of *Oenothera* and many of those of *Epilobium* and probably those of *Fuchsia* as well are crypthybrids.

8. This condition must be clearly recognized in connection with any investigation in regard to the origin of species based on material of this sort, that is, on crypthybrids such as *Oenothera Lamarckiana*, *O. biennis*, etc.

9. Although there appears to be good evidence that hybridism has been an important cause of the multiplication of species, there seems to be no logical support for the view that it has to do with their actual origin.

HARVARD UNIVERSITY

EXPLANATION OF PLATES XXII-XXV

PLATE XXII

FIG. 1.—Spores and elaters of *Marchantia polymorpha;* ×250.
FIG. 2.—Spores of *Polytrichum commune;* ×250.
FIG. 3.—Spores of *Equisetum hiemale;* ×200.
FIG. 4.—Spores of *E. variegatum*, var. *Jesupi*, a hybrid; ×200.
FIG. 5.—Spores of *Angiopteris evecta;* ×200.
FIG. 6.—Pollen of *Zamia floridana;* ×250.

PLATE XXIII

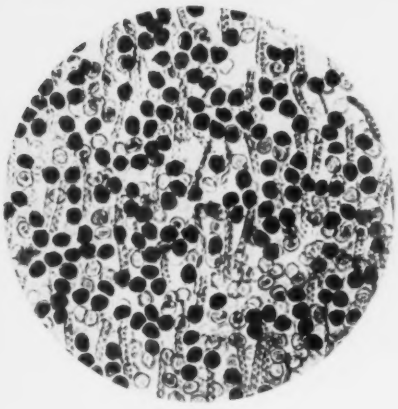
FIG. 7.—Pollen of *Ginkgo biloba;* ×250.
FIG. 8.—Pollen of the same; ×700.
FIG. 9.—Pollen of *Araucaria Cooki;* ×200.
FIG. 10.—Pollen of the same; ×400.
FIG. 11.—Pollen of *Iris versicolor;* ×150.
FIG. 12.—Pollen of *Iris germanica* hybrid; ×150.

PLATE XXIV

FIG. 13.—Pollen of *Lilium canadense;* ×125.
FIG. 14.—Pollen of *Narcissus* hybrid; ×250.
FIG. 15.—Pollen of *Lachenialia Nelsoni*, cultivated hybrid; ×250.
FIG. 16.—Central portion of flower of *Chamaenerion angustifolium;* ×25.
FIG. 17.—Part of flower of *Epilobium hirsutum;* ×25.
FIG. 18.—Anther of the same; ×125.

PLATE XXV

FIG. 19.—Part of anther of garden *Fuchsia* (hybrid of *F. magellanica*); ×200.
FIG. 20.—Part of anther of *Oenothera Lamarckiana;* ×200.
FIG. 21.—Flower of *O. biennis;* ×25.
FIG. 22.—Anther of *O. biennis;* ×200.
FIG. 23.—Anthers of *O. Lamarckiana;* ×50.
FIG. 24.—Anthers of *O. grandiflora;* ×50.



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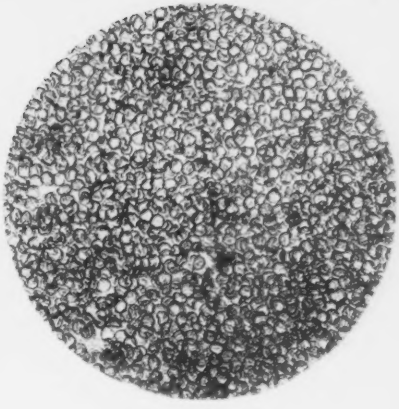

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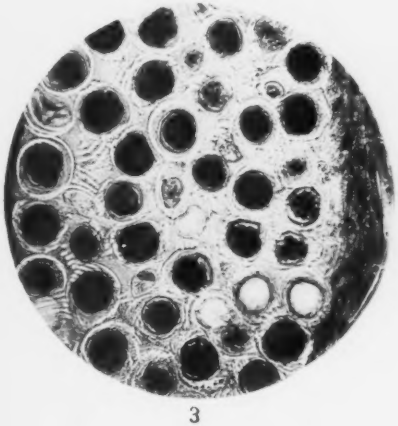

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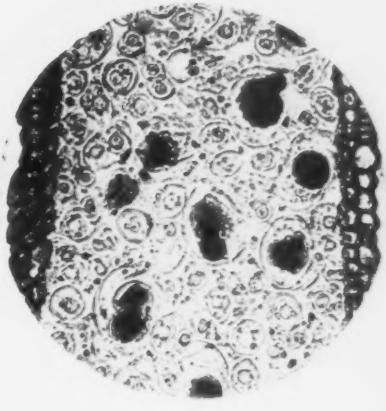

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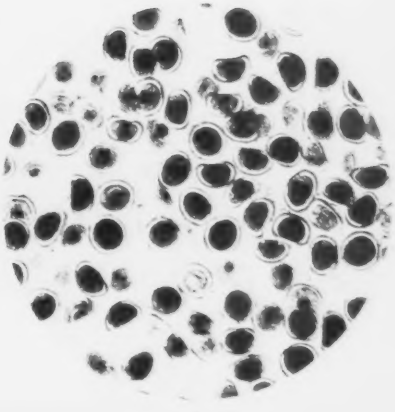

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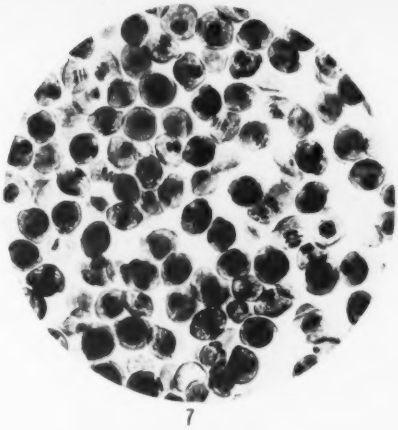
JEFFREY on SPORE CONDITIONS



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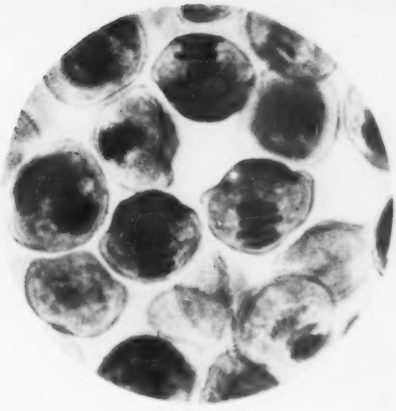

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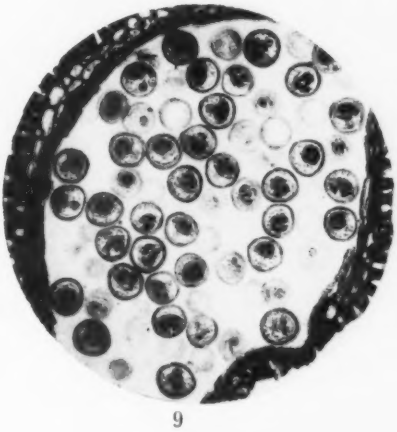

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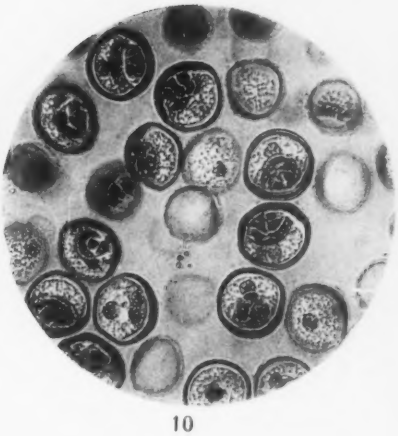

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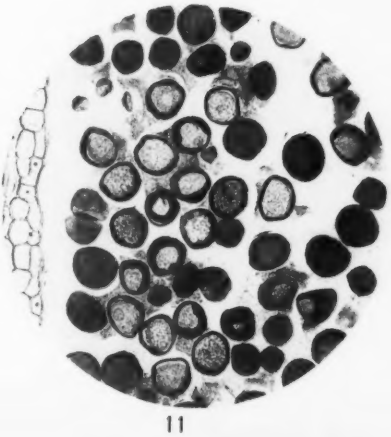

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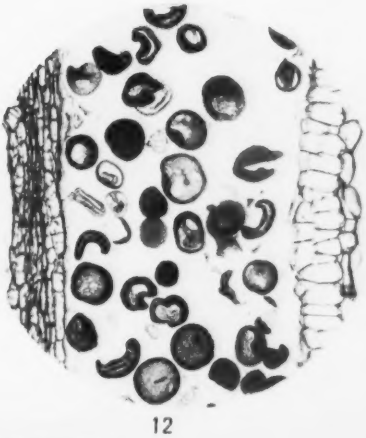

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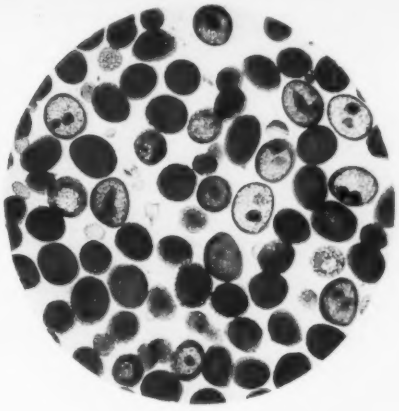

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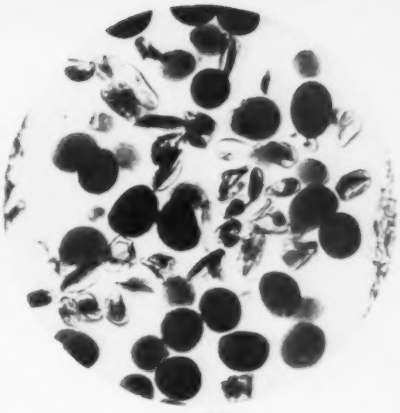

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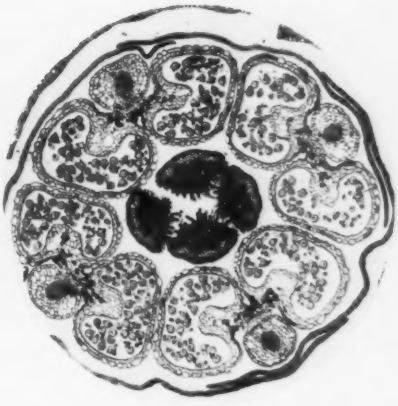

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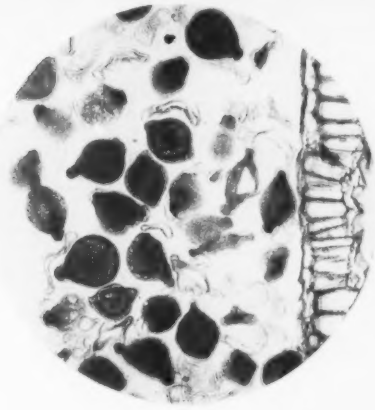

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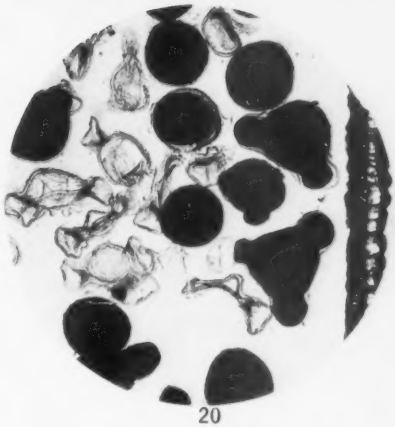

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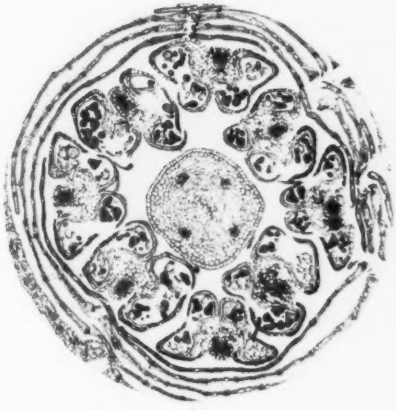

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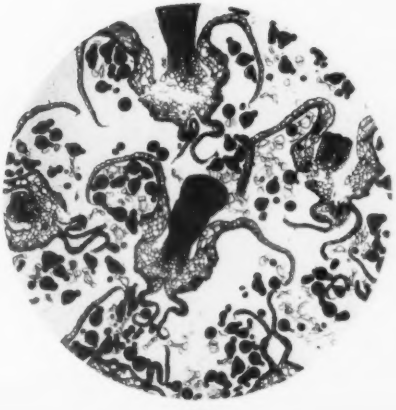

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JEFFREY on SPORE CONDITIONS

REPRODUCTION IN PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 195

JOHN M. COULTER

In connection with the vast accumulation of facts concerning reproduction, our morphological categories are in danger of becoming too rigid. Emphasis is laid upon secondary features, and the fundamental features common to all reproduction escape attention. An extensive terminology further enforces rigidity of classification, because terminology makes exact definitions necessary. As a consequence, the various so-called "types" of asexual and sexual reproduction are pigeon-holed in our texts as though they had nothing in common. It is the purpose of the present paper to attempt a tentative analysis of the phenomena of reproduction as observed in plants, to eliminate the secondary features that make for too great rigidity of conception, and to discover the facts which are common to all reproduction, which underlie all superficial differences, and which, therefore, relate naturally the various "types." It is hoped that such an analysis may serve, not only to soften morphological distinctions, but also to suggest research that will deal with fundamentals more than with superficial details. In such a presentation it would be impossible and confusing to pay attention to all of the variations recorded. Only the general situations can be included, so that the conclusions reached will not seem to satisfy every case, but the point of view will be indicated which can eventually adjust all details.

The use of the term "reproduction" in this paper must be defined. Any cell that produces another one is performing the function of reproduction, but the result may be merely the growth of an individual, the restoration of wasted tissue, or the healing of a wound. Reproduction will be used as meaning the production of new and independent individuals, which is the current understanding of the word.

Vegetative multiplication

In one-celled plants the individual and the cell are identical. Among the varied activities of the protoplast, the power of self-division is one, and in such plants this division results in two new individuals. In other words, this is reproduction, and the conclusion is that the fundamental mechanism of reproduction is cell-division. There is nothing more in the machinery of reproduction than this phenomenon of cell-division, whether it be sexual or asexual reproduction. If the essential machinery of reproduction is provided for in cell-division, it follows that the cell-fusion connected with the sex act must be regarded as an addition to the fundamental process of reproduction, an added preliminary process, not necessary to reproduction, but securing something in connection with it. In fact, the extent to which asexual reproduction occurs among plants is not fully appreciated. It is probably true, taking the plant kingdom as a whole, that the multiplication of individuals is greater by asexual than by sexual methods. The abundant asexual reproduction even among angiosperms testifies to the fact that asexual reproduction is not even a declining method. What may be called the first stage in the evolution of asexual reproduction, therefore, is represented by those one-celled plants whose only method is ordinary cell-division, which is a function exercised by any protoplast under appropriate conditions.

Reproduction by spores

Among many-celled plants, ordinary cell-division usually does not result in new individuals, but in the growth of the individual. This transition from cell-division resulting in new individuals to cell-division resulting in growth is associated with the establishment of a new form of asexual reproduction. So long as protoplasts are held together by their walls in the continuous framework of an individual, there can be no production of new individuals. There must be detachment from the parent stock as a preliminary to the series of cell-divisions that are to result in the new individual. Among the lower algae this is accomplished in a very simple way. The protoplast detaches itself from the cell wall and

escapes into the surrounding medium. The escaped protoplast (spore) is nothing more than a protoplast acting independently of its wall and of its parent plant. No new powers seem to belong to the escaped protoplast; it begins a series of divisions, but being free from the parent form, the divisions result in a new individual. The difference between such a spore, therefore, and any other protoplast belonging to the parent body is not a difference of power but of opportunity.

Ordinarily the spore-forming protoplast begins a series of divisions before escape, resulting in several spores; but it is obvious that the series of cell divisions is responsible for reproduction, whether the protoplast divides before it escapes, or escapes before it divides. In many fungi the detachment of spores is secured, not by the discharge of protoplasts, but by the abstriction of walled cells from special branches. This "pinching off" a part of the body is merely a detail of separation. The essential fact is that a spore means a protoplast separated from one individual, and capable of producing another individual. The multitude of names applied to spores on the basis of secondary characters has obscured the fundamental idea that belongs to all of them, the idea of a detached protoplast.

It would be interesting to know what conditions determine the separation of a protoplast from its wall and its abandonment of the structure it has made and kept in condition. A possible suggestion may be obtained from the occasional behavior of protoplasts at the inception of conditions unfavorable to vegetative activity. Just as in spore-formation, the protoplasts become freed from the wall, round off, and are entirely detached from the general life of the individual which has produced them. The experimental control of spore-formation in many algae and fungi shows that conditions can be supplied which inhibit spore-formation indefinitely, and other conditions can be supplied which stimulate abundant spore-formation. In general, whatever diminishes vegetative activity favors spore-formation, so that it is the physiological condition of the protoplast that determines whether it continues to do vegetative work, or becomes a spore. It is certainly suggested that the favorable condition for spore-formation in nature

is the waning activity of the protoplast. The conclusion is that spore-formation is a response to relatively unfavorable conditions on the part of plants whose activity extends through a period long enough to encounter varying conditions. This would make spore-formation possible among one-celled plants, as well as among many-celled plants.

One of the problems of reproduction, perhaps to be regarded as the most fundamental one, has been suggested by the previous statements. When protoplasts are detached from a parent plant, whether it be a single protoplast called a spore, or a group of protoplasts, as in the case of a propagating bud, the whole structure of the parent is reproduced. Of course the essential separation is physiological, which may take place although complete structural separation has not been effected. When one considers the reproductive power in a group of active cells isolated from the parent stock, such as gemmae, propagating branches, buds, tubers, bulbs, cuttings, isolated nodes, and even leaf fragments, it becomes evident that reproduction is a function of every active cell, and that it can express itself when certain conditions are supplied. The conspicuous condition seems to be detachment from the parent stock. It is really a restoration of lost parts, and therefore is a form of regeneration, differing in degree but not in kind from what is ordinarily called regeneration. From this point of view, a spore is to be regarded not so much as a specialized reproductive cell, differing in power from other protoplasts, but as a single detached protoplast rather than a group of them, and therefore regenerating all the structures of the body. What induces these detached protoplasts, whether single or in groups, to produce a new individual is no more clear than what induces a fertilized egg to produce a similar structure, but both seem to belong to the same category, and that is, a series of divisions that result in a definite structure.

A further stage in the evolution of spore-formation is the differentiation of sporangia, which needs no discussion in this connection. Sporangia are formed under the conditions that favor spore-formation, and not during maximum vegetative activity. They are not formed as parts of the body, awaiting appropriate conditions for spore-formation. The fundamental difference

between plants without and with distinct sporangia is that in the former all protoplasts may respond to the conditions for spore-formation, while in the latter only a restricted number of protoplasts respond. What determines the selection is a question that remains for physiology to answer. When the differentiation of sporangia first takes place, the vegetative cells have either lost the power of spore-formation once common to all vegetative cells, or are inhibited from expressing it. Spore-formation by cells ordinarily vegetative occurs often enough to assure us that spore-formation is only inhibited in such vegetative cells.

A summary of what may be called three stages in the history of asexual reproduction, as given above, may be stated as follows. The first stage is represented by cell-division, which belongs to cell-activity in general; in other words, it is a process as natural to all protoplasts as any work. The second stage is represented by spore-formation, in which ordinary vegetative cells under certain conditions produce spores. In this case the activities of a cell are differentiated by varying conditions, and are not differentiated permanently. The third stage is represented by the reproduction of spores by special cells which are differentiated in function permanently from the ordinary vegetative cells. This specialization of certain cells is accompanied by the inhibition of the spore-producing power of cells in general.

The origin of sex

If all plants were sexual, the origin of sex would be as obscure a problem as is the origin of life. Fortunately for this problem, the most primitive plants are sexless, and the gametes are seen to be as definitely related to previous structures as are any other features of evolutionary progress. What is ordinarily referred to as the "origin of sex," however, is simply the morphological origin of gametes, the visible structures associated with sex. The use made of *Ulothrix* as an illustration in this connection is familiar to all botanists. The gradations from zoospores to gametes are complete, so that it seems to be clear that in this case gametes are morphologically spores greatly reduced in size, and usually incapable of functioning as spores. It is a temptation to infer that

the difference in size means simply a difference in the nutritive capacity, but further consideration shows this to be a hasty conclusion.

The important difference between the spores and gametes of *Ulothrix* is that the latter pair and fuse and the former do not. To obtain any suggestion as to the cause of this difference it is necessary to recall the conditions of spore-formation and gamete-formation. When the conditions favor maximum vegetative activity, neither spores nor gametes are produced. When the conditions are less favorable for vegetative activity, spores are produced; and when the plant is approaching the end of its activity, gametes are produced. It has been found possible to control experimentally the conditions that determine these various activities. The distribution of these functions in the ordinary life history of the plant is naturally related to its changing environment. The production of gametes in the simple plant we are considering is the last act in the life of the plant, an act induced by conditions that are bringing the activity of the plant to its close.

But why do gametes pair and fuse? It is obvious that more important differences than a difference in size have been developed in connection with the derivation of gametes from spores. The difference in size is visible, but in connection with it there develops a very different set of physiological conditions. This first stage of gamete production has been called "isogamy," but the name is only optically true. The same idea is expressed when such plants are said to be "unisexual." Exception may be taken to both of these terms. The gametes are alike in appearance, but that they are not alike in fact is evidenced by their pairing and mutual attraction. Morphological likeness may justify the term "isogamy," but for the term unisexual there is no excuse whatever. Sexuality, with its pairing sexual cells, implies two sexes, whether they can be distinguished or not. All sexual plants must be bisexual to be sexual at all.

The result of the sex act in these primitive sexual plants deserves attention. The zygote produced has the powers of an ordinary spore, in that it can produce a new individual, but the notable difference is that it does not germinate immediately. It is pro-

duced under conditions unfavorable to vegetative activity, and therefore unfavorable for the production of a new individual. The conditions that favor zygote-formation inhibit zygote-activity, and it responds with its heavy wall and dormant protoplast. Even when this zygote germinates, it may not produce a new individual, but the protoplast may divide at once to form spores. In other words, the protoplast of a zygote may function directly as a spore-forming protoplast. The inference is that the production of a vegetatively active individual or the production of spores depends upon the conditions for vegetative activity. If these conditions favor maximum vegetative activity, a vegetative individual will be produced; but if they do not favor maximum vegetative activity, spores will be produced. The succession of conditions at the opening of a growing season is just the reverse of the succession at the ending of a growing season. In the latter case there is a gradually waning activity, resulting in spore-formation following great vegetative activity; while at the opening of a season there is gradually increasing activity, the conditions first favoring spore-formation, and then vegetative activity.

The seasonal relation between spores and gametes lies at the basis of the changes which gradually established a definite alternation of generations. Spores are always as essential a feature of the life history as gametes, and in plants sexual reproduction is never the whole of reproduction.

A summary of the important facts in reference to the "origin of sex" may be stated as follows: (1) gametes have been derived from zoospores which have become so reduced by succcessive divisions as to be incapable of functioning as spores; (2) when first recognized by their behavior, gametes are alike in every visible feature, so that there is no evident distinction of sex; (3) a physiological differentiation of gametes is indicated by their mutual attraction in pairing, so that two sexes are present, although not distinguishable; (4) gametes are formed under conditions relatively unfavorable to either vegetative activity or spore-formation, representing the closing activity of a plant; (5) gametes therefore appear in response to unfavorable conditions that arise in the life history of a plant which is long enough to extend over a considerable

range of varying conditions; (6) the sex act results in a zygote which is formed under conditions unfavorable to vegetative activity, and therefore passes into a protected dormant condition; in other words, the conditions which favor the formation of a zygote inhibit its germination; (7) a zygote may function as an ordinary spore, producing a new individual, or it may produce spores, functioning like an ordinary protoplast; (8) the physiological changes involved by the introduction of the sex act are very great and far-reaching, for they include the mutual attraction of gametes, the organization into a single cell of the contributions of two cells, a provision for reducing the number of chromosomes which the sexual fusion has doubled, and the appearance of two kinds of individuals produced by spores and zygotes.

The differentiation of sex

The differentiation of sex usually discussed is morphological differentiation, which means in this case a visible difference in the size and activity of gametes, so that they can be recognized as male and female. The increase in bulk of one of the pairing gametes is observed to be due chiefly to the increased bulk of cytoplasm, and on this account the egg is said to have much greater nutritive capacity than its mate. This is probably not the only function of the cytoplasm of the egg in connection with the sex act, but whatever the functions may include, they are evidently as subsidiary to the act as are swimming appendages and mutually attractive substances.

Among the heterogamous plants, the variable gamete in appearance is the sperm, and its variability has to do chiefly with its swimming appendages, its form, and its amount of cytoplasm. From algae to gymnosperms, the apparatus for locomotion is developed in a variety of ways, reaching a high degree of specialization in such groups as the ferns and cycads. But among the angiosperms and higher gymnosperms a very different situation has developed, for motility has disappeared, even in its simplest expression. It follows that all the elaborate specialization of the sperm of ferns and cycads has to do with their motility, and not at all with their function as gametes. It is evident also that even the simple cilia

of the primitive gametes are no essential part of a sexual cell. The need for emphasizing this is apparent when it is realized that this secondary feature of a sperm has been regarded as its essential feature by those who demand rigid categories. When motile sperms were first discovered among gymnosperms, they were hailed as the only sperms in seed plants. In other words, the sperms of most seed plants were not regarded as sperms because they cannot swim.

Another rigid conception in reference to the sperms of angiosperms needs attention. Ciliated sperms are produced and discharged by the mother cell. This has led to so rigid a definition of a sperm that if the sperm generation is omitted it is concluded that there are no sperms. The usual formula for describing this situation has been to say that "the mother cell functions directly as a sperm," implying that in fact there is no sperm, but that the mother cell behaves like one. Since the test of a gamete through all its history is its behavior, it is difficult to understand such a statement, except that a secondary feature has been substituted for the essential one. It is obvious that if organization and discharge of a sperm by a mother cell are essential to secure freedom of approach to the egg, when another method of approach is secured, the necessity for discharge disappears. The protoplast within the mother cell and the discharged sperm are the same protoplast. The sperm mother cell of angiosperms behaves like a sperm because it is a sperm.

The obvious conclusion is that a sperm is a protoplast which fuses with another one to form a zygote; that in visible features it differs originally in no essential way from any other protoplast; that eventually it becomes less bulky than its mate on account of a difference in the amount of cytoplasm; that it often develops an elaborate swimming mechanism as a secondary feature; and that the swimming apparatus is eliminated when the necessity for swimming disappears.

The phenomenon of "double fertilization" in angiosperms introduces a situation that is suggestive. In this case a sperm fuses with another cell, so that there is the same mutual attraction as between egg and sperm, leading to contact and fusion, but there

is no production of a new individual. The inference is that the mutual attraction and fusion of two protoplasts is not all that is necessary for reproduction, and that mutual attraction is as much a secondary feature of sexual reproduction as is motility, and simply directs motility. There must be some fundamental difference between an ordinary cell, therefore, and one that has matured as an egg, but at the same time probably any protoplast may mature as an egg. It seems fairly well established that whatever of significance there may be in the sex act is found in the fusion of nuclei. When two protoplasts fuse, therefore, and do not produce a new individual, their nuclei must differ in some way from those of functioning sperms and eggs. One may imagine the adjustment of one nucleus to another before fusion can result in reproduction, and this mutual adjustment probably lies at the basis of sex-reproduction. It also probably explains the fact that sperms and eggs vary in their ability to fuse, and in the results of fusion.

Sexually differentiated individuals

The appearance of male and female individuals may be regarded as the extreme expression of sexual differentiation, which involves much more than the differentiation of male and female gametophytes, with their different sex organs. It is not necessary to present illustrations of the various situations this differentiation includes, for such a presentation would be merely a recital of life histories very familiar to morphologists. When the life histories of sexual plants are considered, ranging from the algae to the angiosperms, the following tentative conclusions are suggested:

Gametes are necessarily differentiated physiologically, and whatever explains this differentiation will explain the sexual differentiation of individuals. It seems to be a differentiation in chemical and physical constitution, which may or may not express itself in bodies visible in the sexual cells.

Whatever may be the cause of sexual differentiation, it is capable of being transmitted through generations of vegetative cells, until conditions favor its expression in the form of gametes and their associated structures. The implication of this statement is that sexuality does not arise *de novo* when gametes appear, but that

what may be called for convenience "sex-determiners" are always present in the plant body. These determiners are conceived of as representing substances that under appropriate conditions react in such a way as to determine the formation and character of the sexual cells.

Along with sex-determiners there must be sex-inhibitors, for it seems clear that every protoplast contains both determiners, but gamete-forming protoplasts produce only one kind of gamete. It seems probable, therefore, that every gamete-forming protoplast is equipped with two determiners and one inhibitor.

In the early history of sex the protoplasts of an individual differ as to this equipment, so that the individual is bisexual so far as gamete-formation is concerned. Later, all the protoplasts of an individual are alike in sexual equipment, and as a consequence individuals are sexually differentiated. Finally, with the appearance of heterospory, sexual individuals are permanently differentiated.

Apparently the amount of available nutrition holds no relation to the differentiation of sex, except as it inhibits the production of highly nutritive eggs by a body of small nutritive capacity, and at the same time permits the production of sperms. In other words, nutrition does not determine sex, but sometimes determines the opportunity for the expression of sex.

All the sporophytes contain both sex-determiners, and in sporogenesis they are transmitted to the spores, which may produce bisexual gametophytes or unisexual gametophytes, dependent upon the distribution of the inhibitors.

All fertilized eggs contain both sex-determiners and inhibitors, and may transmit them all to the sporophyte, in which case the sporophyte would produce spores functioning alike, or two kinds of spores differing in their inhibitors.

In the case of the monosporangiate sporophyte of seed plants, the fertilized egg transmits to the sporophyte only one inhibitor, which determines whether it produces megaspores or microspores.

The presence of sex-determiners and inhibitors determines not only the character of the gametes produced, but also the character of the sexual structures associated with them; and, in the case of

monosporangiate sporophytes, determines the development of a much wider range of structures than the appropriate sex organs.

Parthenogenesis

A strict application of the term parthenogenesis to the germination of an unfertilized egg is intended here. The facts that have accumulated in reference to parthenogenesis among plants seem to justify the following conclusions:

An egg may function as a spore in that it may germinate without fertilization. It seems evident, however, that an egg so differs from a spore in constitution that it needs a different kind of stimulus for germination. Usually this stimulus is applied in connection with the act of fertilization, but it may be applied in some other way.

The peculiar organization of the egg for fertilization is determined at the reduction division. If the reduction division does not occur, parthenogenesis is more likely to occur, and the egg is probably incapable of fertilization.

If spores are eliminated from the life history, as in animals and some plants, reduction occurs in connection with gameteformation.

An egg necessarily produces a sporophyte, and a spore a gametophyte, but vegetative protoplasts of either generation may be organized to produce either generation; that is, they may function as eggs or spores, dependent upon the determiners they have received.

In general, therefore, parthenogenesis differs from reproduction by other protoplasts only in requiring a more specific stimulus, a fact which ordinarily prevents eggs from germinating unless fertilized.

Some conclusions concerning sexuality

Any general survey of the facts connected with sexual reproduction shows them to be very numerous and in some instances apparently contradictory, but they are all consistent with some general situation that determines sexuality. A review of the more prominent facts referred to in the preceding pages may sug-

gest a working hypothesis. There are three features belonging to the most primitive gametes that deserve attention: they are motile, small, and pairing cells.

It is evident that motility is not an essential feature of sexual cells, for early in the evolution of plants, one of the pairing gametes becomes passive, and finally both are non-motile. Motility, therefore, is a secondary feature common to both gametes at first, retained with remarkable persistence by the male gamete, but dispensed with entirely in most seed plants.

It is equally evident that the small size of the primitive gametes, as compared with the spores of the same plant, is not an essential feature of sexual cells. In other words, they are not gametes simply because they are smaller than the spores. Later in the history of plants, one of the pairing gametes becomes much larger than the spores, and still it is a gamete. The difference in size is due chiefly to the varying bulk of the cytoplasm, and in some seed plants the sperm is a naked nucleus. The conclusion is that the amount of cytoplasm is also a secondary feature of sexual cells. It is certainly true that the activity of the cytoplasm of the egg is intimately related to the act of fertilization, not only as a source of nutrition, but also as the source of an activating substance, which LILLIE has called "fertilizin," which determines the physiological moment of fertilization. It should be recognized, however, that even this activating substance is not an essential feature of sexuality, but belongs to the category of secondary features which aid in making the sex act possible.

The pairing of the primitive gametes is certainly a feature that belongs to all gametes, and yet there are pairing and fusing cells that are not gametes. If pairing and fusing are not peculiar to gametes, they do not represent the essential features of sexuality. Pairing seems to be a secondary feature, just as are motility and cytoplasm, and represents a mutual attraction that makes the sex act possible, just as motility is a mechanism that makes pairing possible.

It is certainly true, however, that the primitive gametes differ from the spores with which they are clearly associated in pairing and fusing, and this difference should be accounted for first. Since

spores are formed in conditions of greater vegetative activity than gametes, it is reasonable to suppose that the difference between spores and gametes is due to a difference in the activities of the protoplast in the two cases, a difference associated with declining vigor. Under such conditions, the products of metabolism will differ, and substances will be produced that do not appear when the protoplast is in full activity. This means that gametes will contain substances that spores do not, and among these substances are those that determine the mutual attraction that results in pairing. If this is true, it follows that different substances differentiate gametes into two kinds that are mutually attractive. These characteristic substances which appear later in the vegetative history of protoplasts may be regarded as among the products of waning metabolism. The appearance of different substances under such conditions is familiar in the case of the autumnal coloration of leaves.

It does not follow that these substances which characterize gametes appear only when the vegetative vigor of the plant as a whole is declining. This is generally true of such plants as the filamentous algae, but in more complex plants this decline of metabolic activity may occur in a region of the plant body, rather than in the body as a whole. For example, it is usually stated that the developing sex organs of mosses check the growth of the axis. It is a fair question to ask whether the sex organs do not appear because for some other reason growth has been checked. A checked growth indicates declining vegetative vigor, and this favors gamete-formation. Another illustration of the same fact may be obtained from the appearance of the sex organs of ferns. When a fern spore germinates, metabolism begins in a relatively feeble way, and during this early period antheridia are formed. Later, when the development of the gametophyte becomes more vigorous, sperm-formation may cease. The so-called "inhibitor" of sperms in this case, therefore, is the disappearance of the characteristic substances that belong to gametes, on account of the increasing vegetative activity of the protoplasts. In this case only such gametes can be formed as are characterized by a small amount of cytoplasm. Later in the history of the fern gametophyte, after

it has developed a well nourished body, cells in the apical region become checked in vegetative activity, the substances characteristic of gametes appear again, and eggs are produced.

We still face the fact that there are cell fusions, even pairing and fusing cells, which show the presence of the mutually attractive substances characteristic of gametes, but which do not represent the sex act. All such cases may be explained as evidences of declining vigor of the protoplast concerned, but if pairing and fusing are not peculiar to gametes, although universally displayed by them, what is the esssential feature of gametes, or, in other words, of the sex act? The only answer that can be made is that gametes are pairing cells whose fusion results in the production of a new individual. This means that in addition to possessing mutually attractive substances formed in connection with declining metabolism, gametes possess nuclei so constructed that when the two fuse, a new individual is produced. This does not mean that neither gamete can produce a new individual alone, for parthenogenesis would contradict this. It means ordinarily that a new individual can be produced only after the nuclei have fused. In other words, the essential feature of sexuality must lie in the peculiar structure of the nuclei of the sexual cells. Whether this peculiar structure is chemical or physical, or both, must be a matter of opinion based on no direct evidence. Nor can it be true that gametes are peculiar in containing the factors of heredity, for these must have been handed down through all the cell generations leading to the gametes. Gametes furnish the opportunity for heredity to express itself, but so do spores, and so does vegetative multiplication.

If gametes are peculiar in the construction of their nuclei, how do they develop this peculiarity? The available evidence seems to make it clear that this gametic peculiarity, whatever it may be, is developed in connection with the reduction division. Among plants this division occurs generally in connection with spore-formation, and when there are no spores, as in *Fucus*, it occurs in connection with gamete-formation. In case the reduction division fails in connection with spore-formation, it has been observed that the resulting $2x$ egg is unable to fuse with the sperm. In any event,

the reduction division determines the sexual condition, and the peculiarity of this division, as compared with ordinary division, is related to the peculiar constitution of the nuclei of gametes as compared with other nuclei. If gametes have any structural peculiarity, it must be developed in connection with this peculiar nuclear division.

In well balanced alternation of generations, as in bryophytes and pteridophytes, there are many cell generations between spores and gametes and how the peculiarities of the gametes are transmitted through the cell generations of the gametophyte is a subject of speculation, but certainly something maintains a continuity between spores and gametes. In heterosporous plants, chiefly the seed plants, the cell generations between spore and gamete become fewer and fewer, until finally sperms are reached in two successive cell divisions, and the eggs are reached in one to three successive divisions. The next advance would be the elimination of spores entirely and the occurrence of reduction in connection with gameteformation, as in animals.

In conclusion, the impression one obtains of sexuality as a method of reproduction is that it represents protoplasts engaged in reproduction under peculiar difficulties, that do not obtain in reproduction by spores or by vegetative multiplication, and that its significance lies in the fact that it makes organic evolution more rapid and far more varied.

UNIVERSITY OF CHICAGO

NEW OR PECULIAR ZYGOMYCETES. 3: BLAKESLEA, DISSOPHORA, AND HAPLOSPORANGIUM, NOVA GENERA[1]

ROLAND THAXTER

(WITH PLATES XXVI–XXIX)

Blakeslea, nov. gen.

Mycelium copious, cottony; hyphae very irregular in diameter, the copious branches often rhizoidal and contorted and producing numerous intercalary chlamydospores. Sporangia of two types with numerous intergrading variations: larger solitary sporangia like those of *Choanephora*, with columella and dark appendiculate sporangiospores; and sporangiola without columella, containing few dark appendiculate spores and covering the surface of large spherical sporangioliferous heads to which they are attached by a vesicular basal piece; the heads solitary on the ends of erect fertile hyphae or produced in clusters from the numerous ultimate branchlets which may result from the successive branching of the latter.

Blakeslea trispora, nov. sp.—Plates XXVI and XXVII.

Mycelium colorless to bright orange. Fertile hyphae stout, erect, arising laterally or terminally, very variable in size. Sporangia spherical, nodding or circinate, very variable; the larger spherical with a rough granular wall, the columella from hemispherical to more elongate; the smaller more conspicuously and coarsely roughened, as is often the distal end or more rarely the whole of the sporangiophore, with spores often distinctly larger and the columella frequently obsolete. Sporangioliferous heads solitary, with fewer, twelve or more, sporangiola; or typically in groups of ten or more each bearing forty or more sporangiola and terminating branchlets of the subdichotomously branched extremity of the fertile hypha, the branches and branchlets of which are

[1] Contributions from the Cryptogamic Laboratories of Harvard University, LXXV.

marked by irregular successive constrictions; the sporangiola typically three-spored, rarely four or six-spored, broadly elliptical, falling from the small nearly spherical vesicle which bears them, or, if not fully matured, carrying the latter with them. Spores from all types of sporangia purplish to reddish brown, marked by sub-labyrinthine longitudinal ridges and furrows, oval or long-elliptical, those from the sporangiola at first somewhat three-sided from pressure, bearing a group of straight fine radiating appendages from either pole. Spores very variable; those of the larger sporangia 8–10×4 μ; from smaller sporangia 10–13×5–8 μ; from sporangiola, average 12×5 μ. Larger sporangia 40–50 μ; smaller to 16 μ; sporangiola, average 11–12×13–14 μ; the vesicle 3–3.5 μ. Chlamydospores very variable, average 17–24×8–18 μ.

This interesting type, which has been named in honor of Professor A. F. BLAKESLEE in recognition of his brilliant researches on the Mucorales, appeared as an impurity in a transfer of *Botrytis Rileyi* which was kindly sent me several years ago, together with specimens of the affected larvae, by Professor FAWCETT. The larvae attacked by the *Botrytis* were found on cowpeas at Gainesville, Florida, and it seems probable that the spores of the present fungus, which may have been growing on the faded flowers of this plant, were accidentally transferred to the diseased insect.

The fungus fruits readily on various agar media and does not appear to lose its power of spore production, like species of *Choanephora*, after continued cultivation on nutrients. When first cultivated, the fatty protoplasm of the hyphae was usually bright orange yellow, so that the mycelium as a whole was strikingly colored; but after continuous culture for several years on potato agar, this characteristic has disappeared, or the mycelium retains only a slightly yellowish tint. The mycelium grows very rapidly, and in two or three days the sporangiola begin to be produced in abundance, each fertile hypha bearing from one to a dozen sporangioliferous heads, which under a hand lens resemble exactly in color and appearance the ordinary "conidial" fructifications of *Choanephora*. Instead, however, of producing a primary head from the surface of which the conidial heads arise, as in the last mentioned genus, the fertile hypha branches repeatedly in an irregularly dichotomous fashion, the outline of the branches and branchlets

being characteristically corrugated through the presence of successive constrictions, the ultimate divisions bearing the sporangioliferous heads. From the whole surface of the latter buds arise, distributed without great regularity, which as they develop become distinguished into two parts: a short stout basal stalk which later assumes the form of a nearly spherical vesicle, and a terminal rounded portion which becomes transformed into the broadly elliptical sporangiolum (figs. 3 and 4). In each sporangiolum three spores are normally produced by longitudinal radiate cleavage, but even in typical sporangiola instances occur in which one or all of these spores may divide before maturing, so that there may be four to six in the mature sporangiolum (figs. 5 and 7). In such cases the spores are more irregular in form, size, and arrangement. When fully mature, each sporangium becomes separated from its vesicle (fig. 6); or, if not quite matured, may be separated with the vesicle coherent, as in figs. 5 and 7. Ordinarily the vesicles are left attached to the sporangioliferous heads as is shown in figs. 2 and 3. The normal spores (fig. 9) are somewhat bean-shaped at first, tending to assume a more rounded form after they are freed from the sporangiolum, rather rich purplish brown in color, marked by longitudinal depressions which appear as fine dark lines, more or less parallel and slightly labyrinthine, which, when the spores are freshly discharged in water, are sufficiently conspicuous, although they become faint after swelling or mounting in glycerine. When viewed end on, as in fig. 8, the furrows give the margin a finely corrugated outline. From either pole in all the spores a variable number of very fine and hardly visible, irregularly divergent appendages radiate, which seem to be formed from an intersporal substance and converge to a more or less distinct cap or small mass of viscous material, which sometimes forms a distinct dark spot. These appendages are straight and very fine, seldom much longer than the spore, and are readily seen only in freshly separated spores and with considerable magnification. They seem to be entirely similar to the corresponding structures which are found on the sporangiospores of *Choanephora*. The spores germinate rapidly in nutrients by producing a terminal or lateral germ-tube which grows directly to a mycelium.

Under ordinary conditions sporangiola of the type just described are formed almost to the exclusion of other forms of sporangia; but, especially when the culture is made in a saturated atmosphere, typical sporangia appear, associated with them, having the normal structure of the ordinary sporangia of the Mucoraceae, and similar to those which occur in *Choanephora*. These sporangia, however, are subject to very great variation, and almost every imaginable intermediate condition between a form such as is represented in fig. 16, and the three-spored sporangiolum may be met with. These single sporangia when well developed, as in the instance just referred to, possess a typical columella, and contain very numerous spores, which are more uniform and usually smaller than such as occur in smaller sporangia. Their characters are in general similar to those of the spores formed in the sporangiola; they possess the same appendages, which are perhaps slightly longer and are similarly though less distinctly marked. Their color is often more reddish brown, but is variable. They may rarely be borne almost erect, but are usually nodding or strongly circinate, the termination of the sporangiophore in many cases being coiled in a more or less irregular spiral. Such large forms with small spores are far less frequent than smaller sporangia which show the greatest variability in size (figs. 13–15 and 17), some of which may be hardly larger than the typical sporangiola and like them may possess no columella (fig. 14). On the other hand, abnormalities or more simple conditions of the sporangiolum type of fructification occur which, like that illustrated in fig. 12, serve to break down any clean-cut distinction between the two types. The spores of the smaller sporangia are likely to be larger than those of better developed examples (figs. 13–15), and the walls are more coarsely roughened. The wall of the sporangiophore, and even sometimes of hyphae which bear sporangiola, may be roughened by a scaly incrustation, sometimes confined to the summit, sometimes extending to the base.

Chlamydospores are produced usually in enormous numbers, especially under unfavorable conditions, and vary considerably in form, from cylindrical to nearly spherical.

Although no "conidia" appear to be produced by this species, it is evident that it must be regarded as very closely related to

Choanephora, the peculiar characteristics of its sporangiospores being in themselves sufficient evidence of this relationship. But the chief interest which attaches to it rests upon the fact that the conidia so characteristic of Choanephorae are here replaced by sporangiola similarly related to large spherical heads; and further that these sporangiola, in the life history of one and the same species, pass by almost insensible gradations to large typical solitary sporangia such as are produced normally by a species of *Choanephora*. If, however, one compares the conidial fructification of *C. cucurbitarum*, for example, with the sporangiolate fructification of the present type, one is unavoidably impressed by the close correspondence between them, both in the form, peculiar color, and ornamentation of the spores, and in the similar origin of conidia and sporangiola from large spherical heads. The conclusion seems almost inevitable that in the conidia of *Choanephora* we are dealing with a condition in which the sporangiola of the present type are replaced by single spores which have been called conidia, but which should in all probability be regarded as monosporous sporangiola. On this supposition it might even be questioned whether the two types should be separated generically.

The writer has been unsuccessful in attempting to separate mechanically an outer thin wall from the colored and ornamented wall of the conidia in *C. cucurbitarum;* but that such a thin wall, corresponding to the sporangiolum wall, may actually be present is suggested by the fact that sometimes in normal heads, but more often in those in which the spore formation has been arrested or is in some way abnormal, one finds a condition like that represented in fig. 18*A*. In such instances the contents of the "conidium" has contracted away from the base of the thin colorless mother cell wall, and has surrounded itself with the characteristic purple "conidial" wall, which is clearly distinguished from the empty space below it, as is indicated in the figure cited. No indication has been seen, however, of the formation of more than one spore in this supposed sporangiolum. In all the species of *Choanephora*, moreover, the conidial heads when not primary arise from a primary head, not from a subdichotomously branched hypha-termination as in the present instance, and the distinction between sporangia and

monosporic sporangiola, if we may so call them, is wide and abrupt.

In the type represented by *Cunninghamella* it has been assumed that sporangia of the normal type are wholly lacking; but although none has been seen by persons who have cultivated any of the three described species of this genus, it by no means follows that they may not exist. In *Choanephora*, where they are known to occur, it is often extremely difficult to obtain them, unless under special conditions of cultivation; and in the case of *C. cucurbitarum*, for example, one might continue cultures under ordinary conditions for an indefinite period without ever obtaining any but the conidial form. On the other hand, at least one species of *Choanephora* is known to the writer, which was isolated by BLAKESLEE during his investigations on the Mucorales, and has never been seen to produce anything but normal sporangia with the typical appendiculate spores of this genus, although it was kept in cultivation for a period of years. As in this instance it cannot be assumed that "conidia" do not exist, so also in the case of *Cunninghamella* it remains to be determined whether sporangia of some sort are not occasionally produced under special conditions. In whatever way this question may be settled by further investigation, it is evident that the "conidia" of this genus, which are also borne on heads having the characteristics of the form-genus *Oedocephalum*, may, like those of *Choanephora*, and with equal plausibility, be considered homologous with the sporangiola of the form under consideration.

In a recent paper on the reproduction of the Mucorales, MOREAU (Le Botaniste **13**: nos. 1–3) has advanced the view that heads of the *Oedocephalum* type represent sporangia which have, as it were, been turned inside out; and that the sporangiospores thus exogenously produced are to be regarded as conidia. He would then trace to the sporangium thus metamorphosed all *Oedocephalum* conditions, isolated instances of which are known to occur among the higher fungi, *Polyporus annosus*, *Corticium effuscatum* in the Basidiomycetes, and species of *Aleuria* among the Discomycetes. The spores endogenously formed in the clavate or cylindrical bodies, which in *Syncephalastrum* replace the single spores of *Cunninghamella*, MOREAU also regards as conidia, and not as

sporangial spores, comparing them to the phaeophragmic spores of *Thielavia basicola*, in which the endospore may become more or less separable from the exospore before the disunion of the spore-segments. To the writer such an explanation seems highly improbable, nor does the presence of the *Oedocephalum* type in diverse and scattered instances among the higher fungi seem in the least significant in this connection; but rather as an indication that this simple and effective method of economizing space and raising a large number of spores above the substratum is a convenient type, which has been used independently by various unrelated groups, even by the Peronosporales.

The spores of *Syncephalastrum* appear to arise by internal, not always transverse, cleavage, rather than by transverse septation as in the phragmospores of *Thielavia basicola*, and the bodies which contain them are in the writer's opinion true sporangiola, from which it is a very short step to the "conidia" of *Cunninghamella*, which, as has been suggested, might be regarded as monosporic sporangiola. That monosporic sporangia are not an anomaly in the Mucorales is shown by the characters of the genus described below as *Haplosporangium*. This opinion seems further substantiated by the comparison already made between *Choanephora* and the present genus, the sporangiola of which are, as has been pointed out, but a short step from the "conidia" of the former, and are clearly shown to be sporangial in their nature, not only through the presence of intermediate forms, but by reason of their peculiar appendages so characteristic of sporangiospores in the Choanephorae.

In a majority of the other "Cephalideae" the conditions are superficially, though not fundamentally, different from those seen in *Syncephalastrum*, owing to a different arrangement for spore-dissemination. An intersporal substance is here present which causes the sporangiospores to cohere at first, and to separate only when this substance together with the sporangium wall becomes transformed to a somewhat viscous fluid; which, as in species of *Syncephalis*, causes all the spores formed on a given head to become united in a large viscous droplet, which adheres to small Drosophilidae and other insects frequenting the substrata on which they grow. In *Syncephalastrum*, on the other hand, the spore-mass when

ripe is quite dry, and spore-dissemination is probably for the most part accomplished by air currents. It is hardly necessary to mention that both these methods of dissemination occur, and may be associated, in other genera of the Mucorales, like *Mucor* and *Mortierella*.

In this connection it may be mentioned that since the publication of the writer's note on *Choanephora* (Rhodora **5**:97) the common *C. cucurbitarum* has been cultivated in the Harvard Laboratories from Cuba, Porto Rico, Venezuela, and other localities, and that the culture, formerly mentioned as having been brought by the writer from La Plata, Argentina, which seemed to correspond to this species, has been contrasted with the plus and minus strains separated by BLAKESLEE from New England material of this species. As a result, the Argentine form produced abundant conjugations, forming perfect zygospores, which left no doubt as to its identity. The normal sporangia were also obtained by subjecting these forms to special conditions of moisture and nutrition, and were found to agree in all essentials with the figures and description given by MOLLER of his *C. americana*. Since, therefore, the ranges of the two overlap, and there seem to be no essential differences between them, the synonymy suggested in the *Rhodora* note may be regarded as finally established.

In addition to *C. cucurbitarum*, the writer has had in cultivation for some years two other American species. One of these was first obtained on *Hibiscus* flowers in the park at Palermo near Buenos Aires, and appears to correspond to *C. infundibulifera*, the secondary heads of which, unlike those of *C. cucurbitarum*, are persistent and become cupulate after the spores, which are smooth, have been shed. This species is very common on *Hibiscus* flowers in the West Indies, and is everywhere abundant in Grenada and Trinidad during the rainy season, where it was often seen by the writer fruiting abundantly on flowers which were still attached to the host plant, or even on buds that had apparently been hindered from opening by its growth. It has also been cultivated on material received from British Honduras and from Guatemala.

A third species, unlike either of the two just mentioned, was also found on flowers of Cucurbitaceae near Buenos Aires and was

kept in cultivation for eight years. Unfortunately, the culture ran out during the writer's absence in the West Indies. Although allied to *C. cucurbitarum*, this species differs in possessing smooth spores of a lighter red-brown color.

Dissophora, nov. gen.

Fertile hyphae abruptly differentiated from slender creeping vegetative filaments, stout, of indeterminate growth, and producing sporangiophores which arise as buds, continuously produced, behind its advancing apex. Sporangia and sporangiophores as in *Mortierella*.

Dissophora decumbens, nov. sp.—Plate XXVIII

Primary fertile hypha at first erect, then decumbent, stout, creeping over the substratum indefinitely, rarely septate, sometimes producing short branches; the sporangiophores arising irregularly from all sides immediately behind its growing apex, tapering somewhat at the base and apex, often septate when mature, rarely branched. Sporangia spherical, columella none, spores spherical or irregularly somewhat angular from pressure; the whole clear white at first, the sporangia becoming slightly yellowish. Primary fertile hyphae up to 10 mm. or more in length, 10–16 μ in diameter. Sporangiophores 100–150×8 μ. Sporangia 17–23 μ. Spores about 4 μ.

On dung of wood mouse from vicinity of Cambridge.

This species was cultivated for several years, but like the two succeeding forms was unfortunately lost during the writer's absence from Cambridge, so that the accompanying figures and description have been made from mounts and dried material. Although very closely allied to *Mortierella*, the peculiarities of the primary fertile hypha, which is unlike that of any others of the Mucorales, has seemed sufficient basis for generic separation. The fructification is pure white and easily distinguished among other fungi with which it may be growing. At first it is erect, but soon becomes decumbent, the older prostrate axis being left behind by the constantly advancing apex (figs. 19 and 26), and reaches a considerable and

more or less indefinite length. Immediately behind the blunt stout apex scattered buds arise (fig. 26), which soon become clavate branches, slightly divergent or even appressed, and as one traces these branches backward from the tip a gradual transition is seen from the first buds to the fully matured sporangiophores (figs. 19 and 20), and when the older portions of the axis are reached, the old sporangiophores alone are found from which the sporangia have disappeared (fig. 21). In a few instances the sporangiophores have been seen to be replaced by a branch on which two or several sporangiophores may be borne. As the latter become fully mature, and when the spores are ripe, one or more septa usually make their appearance and may also occur in the older parts of the fertile axis at considerable intervals. The latter originates from very slender hyphae running on the substratum and are very abruptly distinguished from them (fig. 25). Whether the mycelium is parasitic as it grows in nature has not been determined, but it develops very readily on potato agar without the presence of any host.

Haplosporangium, nov. gen.

Mycelium of slender branching filaments forming a felted layer on the surface of which numerous intercalary or terminal portions become differentiated, forming a superficial network of stout, often septate, segments, or series of segments, of variable length; from which sporangiophores are radiately produced, of characteristic form, broad at the base and attenuated distally to a threadlike termination which bears the primary sporangium and may be subtended by one, rarely two, threadlike secondary sporangiophores. Sporangia monosporous or bisporous.

The species of this genus differ from those of *Mortierella*, to which they are closely allied, in the presence of highly differentiated often very long segments, from which arise peculiar sporangiophores bearing threadlike terminations or lateral branches, on which are produced minute sporangia containing usually only one, sometimes two, spores. The sporangiferous segments are very characteristic and variable, forming a network on the surface of the mycelial weft, crossing it in all directions, and lying parallel to the substratum; sometimes a mere swelling from which a single spo-

rangiophore is produced, but often elongate and bearing large numbers of the latter which arise from all sides without regularity.

The genus resembles *Dissophora* in possessing specialized structures from which the sporangiophores arise, but is clearly distinguished from the fact that these structures are intercalary and determinate, as well as by its peculiar sporangia. In general appearance, and in its habit of producing its sporangiophores in a radiate fashion from the segments of repent filaments, it closely resembles certain hyphomycetous forms like *Hyalopus* or *Cephalosporium*, and it was at first mistaken for an imperfect fungus of this nature when examined with a hand lens. The sporangia are very minute, even when they contain two spores, and the plant would hardly be taken for one of the Mucorales unless examined with some care. The presence of numerous septa in the sporangiferous segments also serves to render the general appearance of the fungus deceptive.

Both the species described below have been cultivated on nutrient agar, one of them for nearly twenty years; but, as has been previously mentioned, both were recently lost, and the material now available consists of dried specimens and glycerine mounts. In the accompanying figures no attempt has been made to represent the general appearance of the mesh of sporangiophoric segments which, though very striking in appearance, is too intricate for satisfactory drawing. The segments illustrated are small, but many occur which may reach a millimeter or more in length, with more or less clearly marked constrictions where septa occur. They are rigid and usually very straight, tapering off at the ends to fine filaments which may at once pass into another segment, or branch and run for a considerable distance before this occurs.

Haplosporangium bisporale, nov. sp.—Plate XXIX

Clear white becoming yellowish with age, forming a dense cobweb-like layer on the substratum, composed of slender filaments connecting the fertile segments which form the greater portion of the layer and may be very short, producing only a single sporangiophore, or may reach more than a millimeter in length and produce large numbers of the latter developed at right angles and in all

directions. Sporangiophores simple or rarely furcate, the abruptly attenuated extremity usually straight or but slightly bent, and bearing the primary sporangium; one, or less often two, secondary sporangia borne on short straight fine branchlets which project at right angles, or may be bent slightly downward, and are opposite when two are present. Primary and secondary sporangia minute, spherical, smooth; containing one spherical spore, or less frequently two, which are subhemispherical in shape, becoming somewhat rounded. The spores hyaline, often, but not always, distinctly roughened. Bisporous sporangia 11–12 μ; monosporous 8 μ. Sporangiophores, average, 48–55$\times$5 μ. Hyphae 1–3 μ, segments to 6.5 μ in diameter.

This form was first found on pig dung at Burbank, E. Tennessee, more than twenty years since, and has since been observed at Kittery Point, Maine, on dung of skunk and of field mice, and also at Intervale, New Hampshire, on dung of squirrels.

The species appears to be not very rare, and has probably escaped notice from its resemblance to some very insignificant hyphomycetes of the *Hyalopus* type. Although all the strains examined produce two-spored sporangia, which are in some cultures very abundant, in one instance at least they are very rare, and may be found only after long-continued search among the predominant one-spored type. Both the primary and secondary sporangia may produce two spores without regularity and on the same sporangiophore; a one-spored primary sporangium may even be associated with a two-spored secondary one. Both this and the succeeding species grow readily on rat-dung agar, fruiting abundantly.

Haplosporangium decipiens, nov. sp.—Plate XXIX

Similar to the last. The sporangiophores on the average constantly somewhat larger, the sporangia always one-spored and nodding, the sporangium wall roughened by minute folds, the spores spherical and smooth. Sporangiophores, average 60–65 μ. Sporangia 8–10 μ.

This species, although it has been cultivated from the original material, has been seen but once and was found on dung of the curious cave-dwelling

Selenodon from Hayti. It seems sufficiently well distinguished from the preceding species by its roughened nodding sporangia, smooth spores, which are always solitary in the strain examined, and its slightly larger dimensions. It is not impossible, however, that strains may occur in which two spores may be produced in single sporangia, as in *H. bisporale*.

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EXPLANATION OF PLATES XXVI–XXIX

The figures have been drawn with a camera lucida and reduced in reproduction. The objectives used, Zeiss dry and Leitz water immersion, are indicated in each case, and also the eyepiece numbers.

PLATES XXVI AND XXVII

Blakeslea trispora Thaxter

FIG. 1.—Habit of moderately well developed group of heads bearing sporangiola, showing a portion of the fertile hypha and its origin; D2.

FIG. 2.—Group of heads denuded of sporangiola; D2.

FIG. 3.—Single head with five sporangiola attached, showing corrugation of branchlets below heads; water im. 4.

FIG. 4.—The same in optical section, much younger; the spores not yet mature in the sporangiola; water im. 4.

FIG. 5.—Sporangiolum mature, with adherent basal piece and containing six spores; water im. 4.

FIG. 6.—Normal three-spored sporangiolum; water im. 4.

FIG. 7.—Sporangiolum with four spores, the basal piece adherent; water im. 4.

FIG. 8.—Three-spored sporangiolum seen in optical section from above; water im. 4.

FIG. 9.—Four spores from sporangiola; water im. 12.

FIG. 10.—Chlamydospores; D4.

FIG. 11.—Fertile hyphae from the irregularly swollen end of which five small sporangia were produced intermediate in character between sporangiola and small normal sporangia; water im. 4.

FIG. 12.—Fertile hypha with single head bearing more normal sporangiola; water im. 4.

FIGS. 13–17.—Different variations seen in normal sporangia; water im. 4.

FIG. 18.—Sporangial spores from the sporangium shown in fig. 16; water im. 12.

Choanephora cucurbitarum

FIG. 18*A*.—Two "conidia" showing thin membrane inclosing space below the single spore and suggesting a monosporangiolum; water im. 12.

PLATE XXVIII

Dissophora decumbens

FIG. 19.—Terminal portion of a fertile hypha in two parts, showing origin and progressive development of sporangiophores; A4.

FIG. 20.—Portion of a fertile hypha bearing mature sporangiophores, in which septa have begun to appear; D4.

FIG. 21.—Older portion of same bearing naked septate sporangiophores; D4.

FIG. 22.—Termination of sporangiophore from which sporangium has disappeared; water im. 12.

FIG. 23.—The same bearing a mature sporangium; water im. 12.

FIG. 24.—Sporangiospores; water im. 12.

FIG. 25.—Origin of fertile hypha from vegetative hypha; D4.

FIG. 26.—Terminal portion of fertile hypha showing origin of sporangiophores behind growing apex; D4.

PLATE XXIX

Haplosporangium bisporale

FIG. 27.—Sporangiferous segments, small and of moderate length, bearing sporangiophores or just budding to form them; D4.

FIG. 28.—Small segments each giving rise to one or two sporangiophores and connected by slender isthmuses; D4.

FIGS. 29–32. Sporangiophores bearing one, two, or three one- or two-spored sporangia; water im. 4.

FIG. 33.—Mature bisporangium; water im. 12.

FIG. 34.—Two spores from a bisporangium still adherent; water im. 12.

FIG. 35.—The same separated; water im. 12.

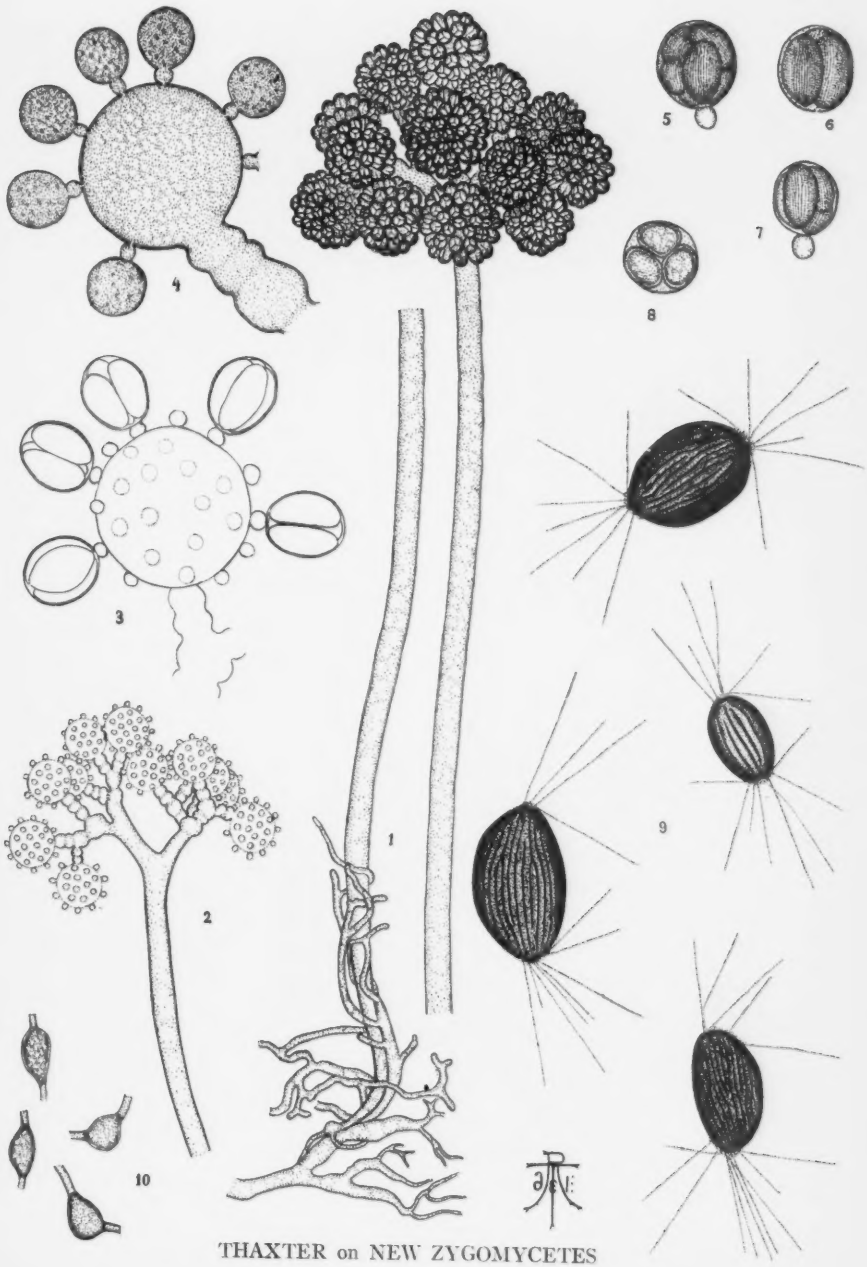
FIG. 36.—Spore from monosporangium; water im. 12.

Haplosporangium decipiens

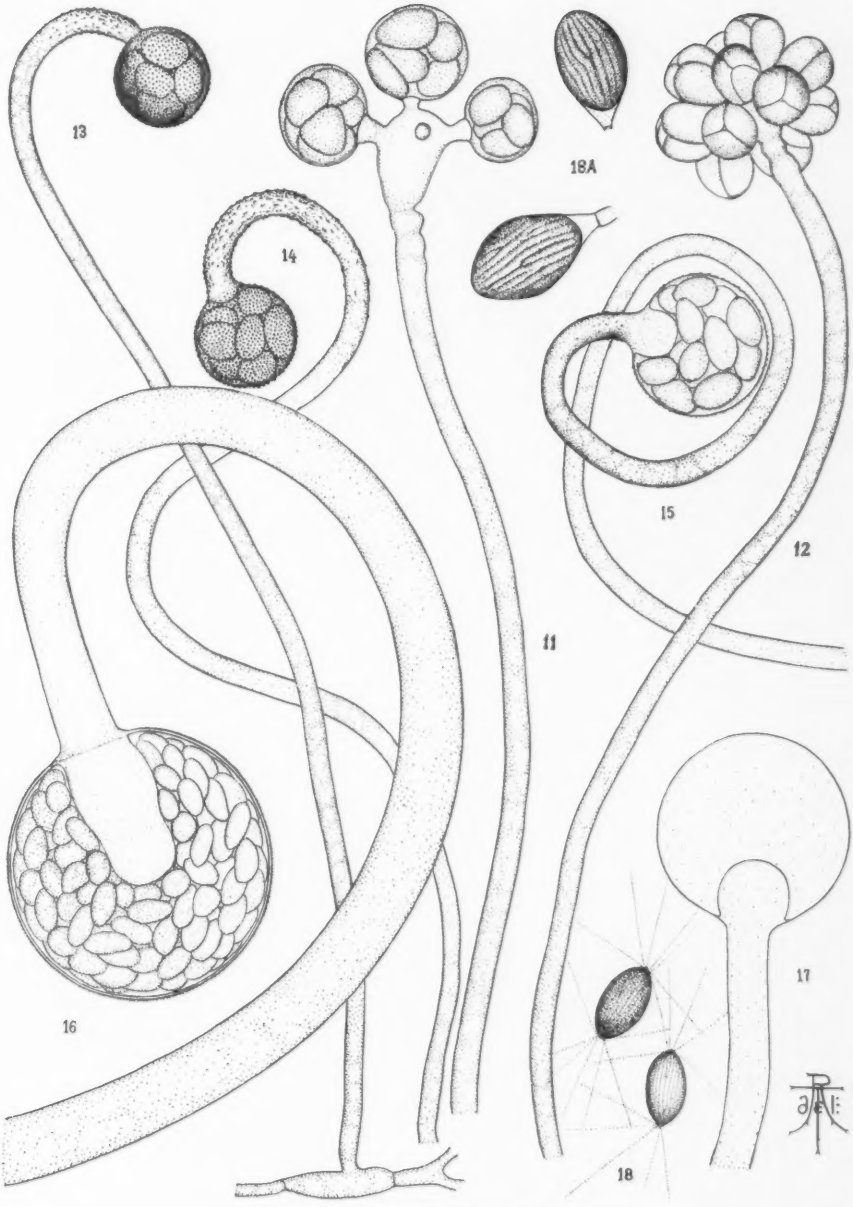
FIG. 37.—Small segments bearing sporangiophores; water im. 4.

FIG. 38.—Sporangiophores; water im. 4.

FIG. 39.—Monosporangium with its single spore; water im. 12.



THAXTER on NEW ZYGOMYCETES



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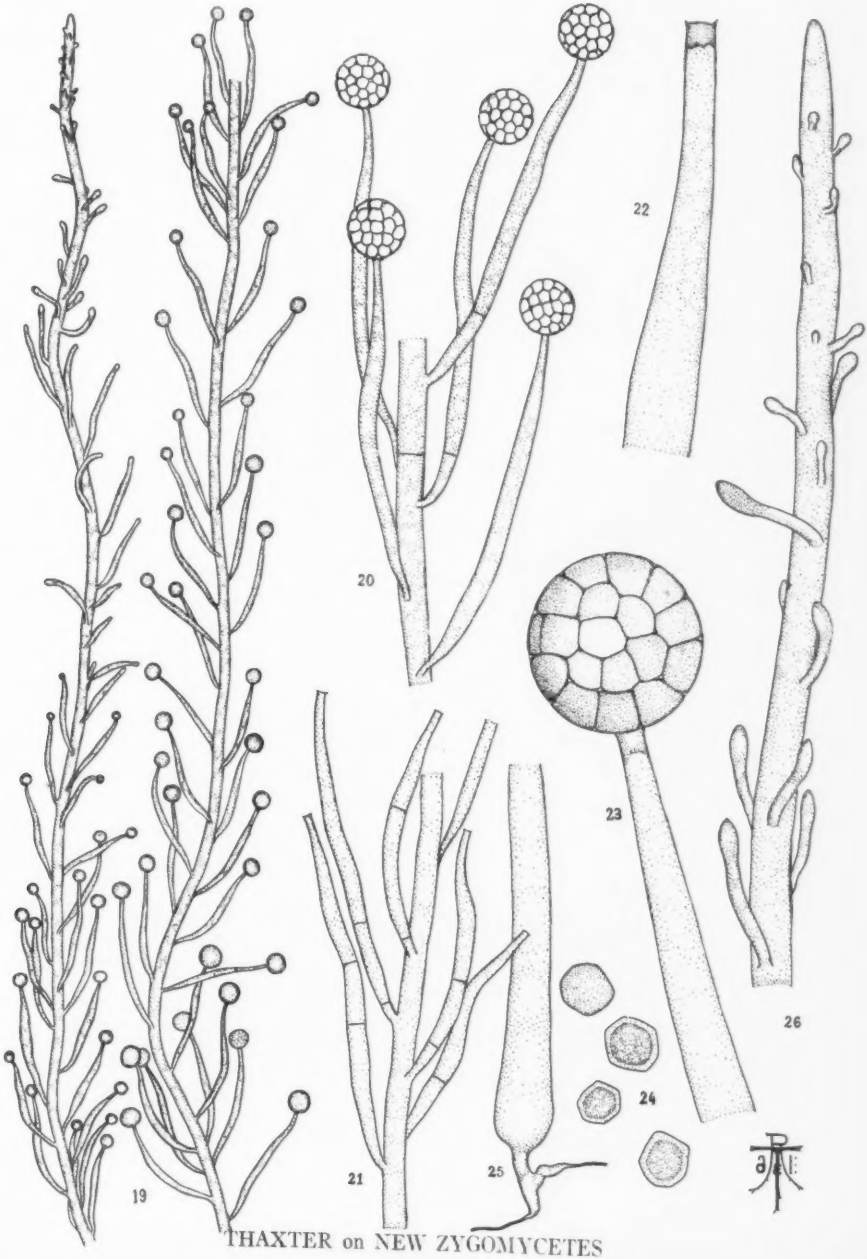

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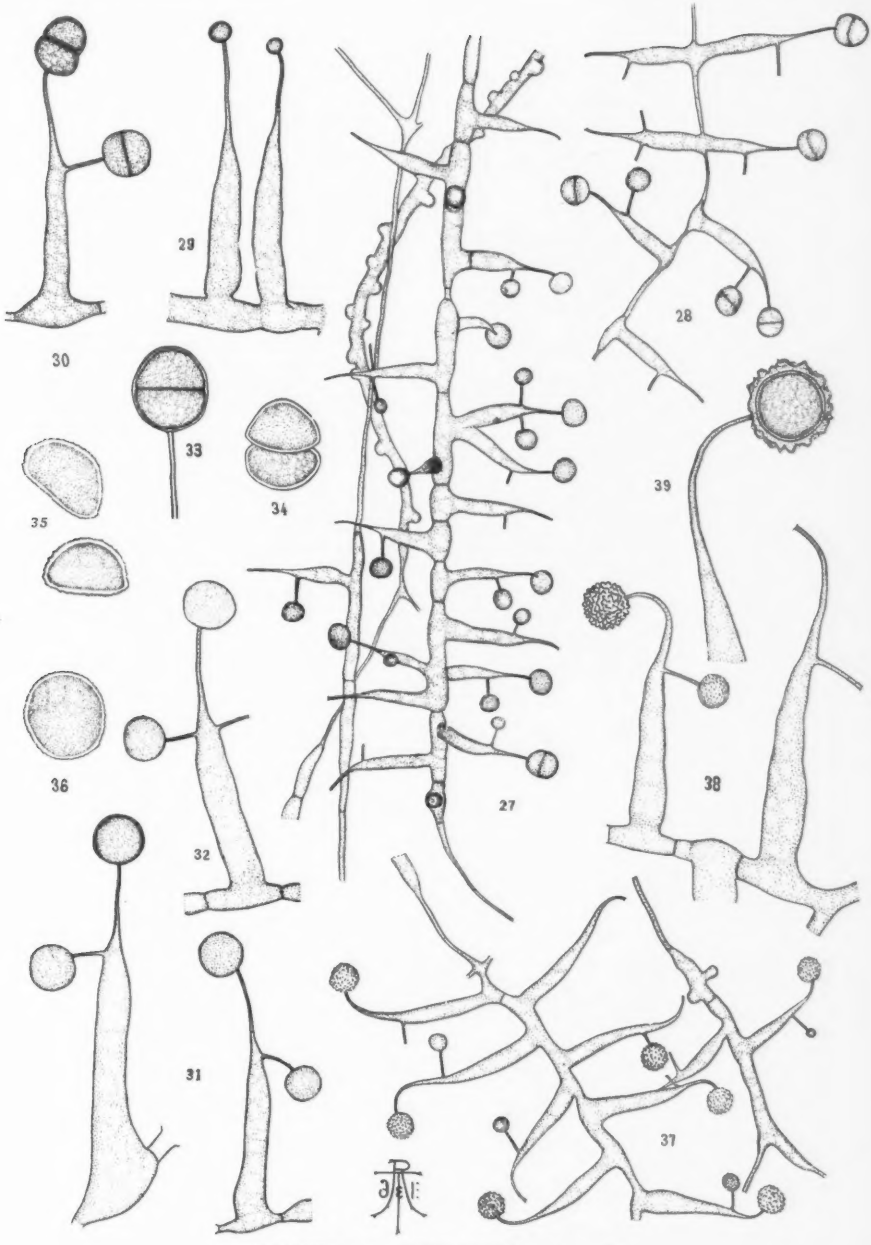

THAXTER on NEW ZYGOMYCETES

THE FORMS OF ANTAGONISM CURVES AS AFFECTED BY CONCENTRATION

W. J. V. OSTERHOUT

(WITH THREE FIGURES)

A study of the literature shows that the effect of concentration on the form of the antagonism curve is not fully appreciated. The alterations in the form of the curve are very marked when solutions are diluted to one-half strength. The writer has found this to be the case with antagonism curves of different types obtained by using a great variety of solutions. The purpose of this paper is not to give the results of these experiments, but merely to state some general principles in the hope that they may be of use to those who have occasion to make quantitative studies on antagonism.

For this purpose curves have been prepared which are diagrammatic composites of the curves obtained by the use of several pairs of salts; these composite curves are shown in fig. 1. For the sake of simplicity they are represented as having been obtained by the use of one pair of salts, which are designated as *A* and *B*. The curve *CDE*, therefore, represents diagrammatically the growth of roots in mixtures of equally toxic solutions of two salts, *A* and *B*. The abscissas represent molecular proportions; thus the point *G* represents a mixture in which the dissolved molecules are 75 per cent *A* and 25 per cent *B;* the point *H* a mixture in which the dissolved molecules are 50 per cent *A* and 50 per cent *B*. The ordinates represent the growth of roots in the various mixtures.

The antagonism at any point is the total growth minus the growth which would have taken place if no antagonism existed.[1] This antagonism is best expressed as percentage of the growth which would have taken place in the absence of antagonism. Hence the antagonism at the point *G* is expressed as $\frac{FD-FG}{FG}\times 100$.

[1] This latter amount of growth is called the additive effect and is expressed by the horizontal dotted line *CE*. Cf. BOT. GAZ. 58:178. 1914.

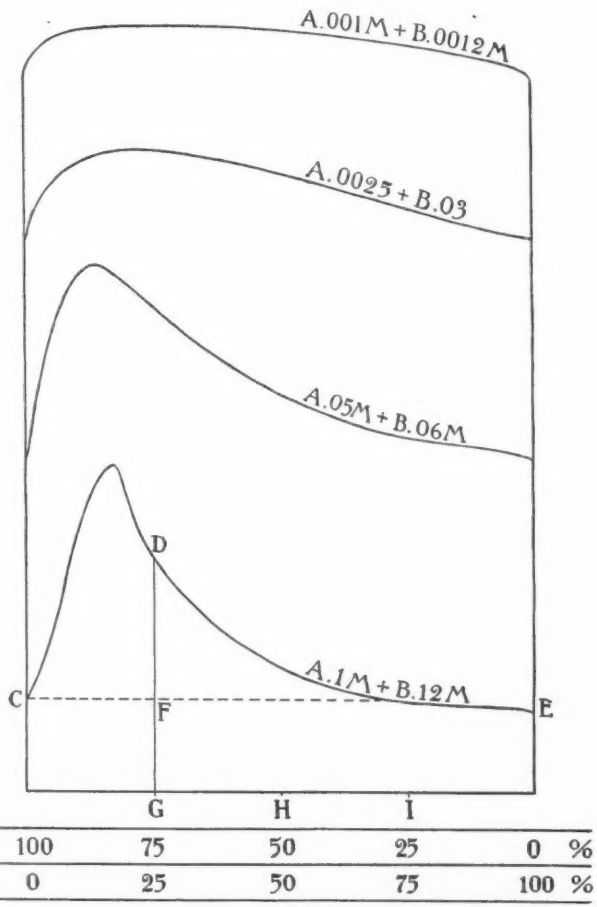

FIG. 1.—Effect of dilution on the forms of antagonism curves: the ordinates represent the growth of roots in solutions, the composition of which is represented by the abscissas; for example, on the curve *CDE* the ordinate at *G* represents growth in a mixture of *A* 0.1 M and *B* 0.12 M in such proportions that 75 per cent of the dissolved molecules are *A* and 25 per cent are *B;* on the curve which lies immediately above *CDE* the ordinate at *G* represents growth in a mixture of *A* 0.05 M and *B* 0.06 M in such proportions that 75 per cent of the dissolved molecules are *A* and 25 per cent are *B*.

The figure shows in a diagrammatic way the effect of dilution on the form of an antagonism curve. The lowest curve *CDE* shows the effect on growth of various mixtures of two equally toxic solutions *A* 0.1 M+*B* 0.12 M. The next curve shows the form of the antagonism curve when all of these mixtures were diluted by the addition of an equal volume of water (*A* 0.05 M+*B* 0.06 M). The next curve was produced by growing plants in mixtures of *A* 0.0025 M+*B* 0.03 M. The topmost curve was obtained with mixtures of *A* 0.001 M+*B* 0.0012 M.

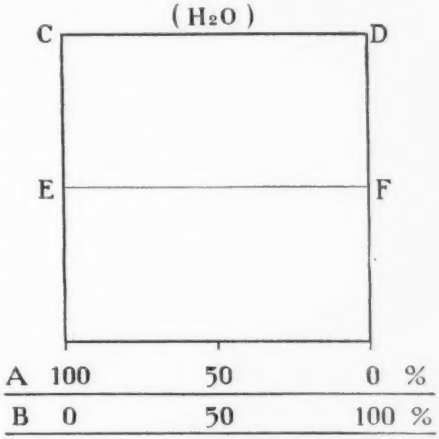

FIG. 2.—Diagram representing the composition of solutions (this serves as the base of the solid model shown in fig. 3): the lowest line represents various mixtures of solutions of two salts, *A* and *B;* the line *EF* represents the same mixtures diluted with equal volume of water; any line drawn parallel to *EF* will express the same mixtures diluted to a degree corresponding to the position of the line, the nearer it approaches to *CD* the greater the dilution; on the line *CD* all points represent distilled water.

The pairs of pure solutions were in each case equally toxic, as is shown by the fact that the two ordinates at the ends of each curve are equal in height.

It will be observed that as the solutions become more dilute, the antagonism curve becomes flatter, and it is evident that at still greater dilutions it must tend to become a horizontal straight line.

In order to give a complete description of the changes in the antagonism curve as dilution increases, it is necessary to construct a solid model. This might have as its base a triangular diagram as described in a previous paper.[2] The apices of the triangle would in that case represent *A*, *B*, and H_2O.

[2] BOT. GAZ. 58:178. 1914.

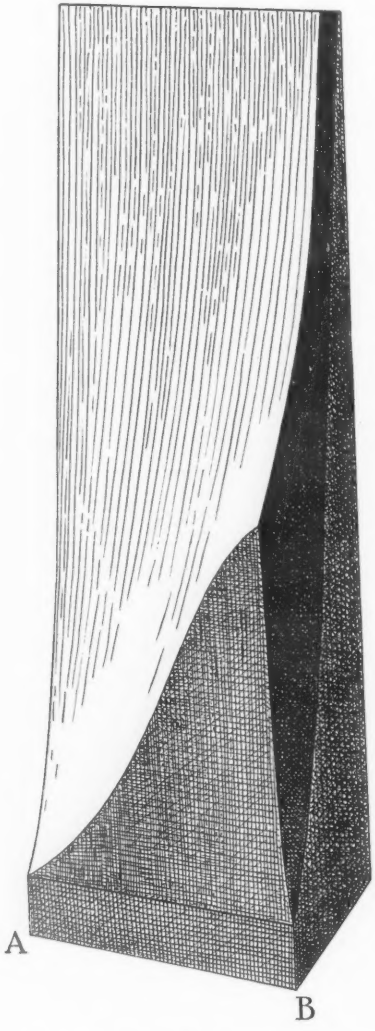

FIG. 3.—A solid model which gives a complete description of the changes produced in the form of the antagonism curve by altering the concentrations of the solutions.

It is more suitable for our present purpose to employ a square as the base and to represent the composition of the solutions according to the scheme shown in fig. 2. In this figure the abscissas have the same significance as in fig. 1, while the ordinates represent various dilutions of the mixtures. Thus all points on the line *CD* represent distilled water, while a point such as *E*, halfway between *A* and *C*, represents a mixture containing equal quantities of distilled water and of *A* 0.1 M. The points on the line *EF*, therefore, represent the same mixtures as the corresponding points on the lowest line, except that the concentrations are in all cases just one-half as great as those represented on the base line. It is evident that the growth in any concentration may be expressed by erecting at the proper point a line perpendicular to the plane of the paper. In this way we may obtain a solid model which gives a complete description of the changes in growth produced

by diluting the various mixtures. Such a model is shown in fig. 3.

It may be desirable to add something concerning the comparative amount of growth in distilled water and in balanced solutions. When dilute solutions are employed, the maximum growth obtained in a fully balanced solution may be greater than that in distilled water, owing to the nutrient action of the solution. But when strong solutions are employed, this is not the case, for the osmotic pressure of the strong balanced solution will inhibit growth.

In general, growth in strong solutions furnishes a much more satisfactory criterion of antagonism than growth in weak solutions. The inhibition of growth due to the osmotic pressure of the strong solutions does not noticeably affect the form of the antagonism curve, since it makes itself felt in the pure solutions to about the same degree as in the mixtures. When we employ the method of mixing equally toxic solutions, the osmotic disturbances are compensated in a manner which has been previously discussed.[3]

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[3] BOT. GAZ. 58:178. 1914.

CURRENT LITERATURE

NOTES FOR STUDENTS

Mitosis in Vicia.—SAKAMURA[1] has studied the somatic and maturation mitoses in *Vicia Cracca* L. In the somatic cells of the young flower organs he has made the following observations. At anaphase the 12 chromosomes, which vary in size, pass to the poles without splitting. After being tightly pressed together, they separate and become vacuolate chiefly along the center, which results in the appearance several writers have erroneously interpreted as a telophasic split. Contrary to the opinion of FRASER and SNELL, the anastomoses are thought to play no important part in the transformation of the chromosomes. The chromosomes can be distinguished as alveolar bands very late, but are no longer visible when the resting stage is fully reached. The resting reticulum is finely uniform, with neither prochromosomes nor other conspicuous chromatic aggregations. In prophase the reticulum gives rise to chromatic bands which have the same arrangement as those in the preceding telophase. This persistence of polarity is believed to favor the theory of the individuality of the chromosomes. There are no chromomeres, no tetrad structure such as Miss MERRIMAN has described, and no continuous spirem either at telophase or at prophase. The split occurs in the very early prophases and remains until the metaphase.

In the pollen mother cells the reticulum is somewhat looser than in the somatic cells. The chromatic granules appear to be more numerous, but there are no prochromosomes. As synizesis approaches, the granules form many small clumps along the linin threads and the whole network contracts into a tight ball. Although a parallel arrangement of threads was not seen before or during the contraction, the author believes such a side-by-side conjugation occurs, since double threads are seen projecting from the knot and in the immediately following stages. As the spirem loosens up, the two halves are distinguishable only in places, but later become very distinct. When the "hollow spirem" is formed they fuse, but soon split apart again. A second contraction occurs, during which the 6 bivalent chromosomes are formed by the simple cross segmentation of the double spirem.

At diakinesis the gemini, 2 of them larger than the other 4, sometimes show a secondary split, thus forming "Längstetraden" such as other authors have described. At metaphase of the first maturation mitosis, the members of each pair appear to be wholly fused. As they separate during the anaphase, the split which is to take effect in the second mitosis appears. At telophase there

[1] SAKAMURA, T., Über die Kernteilung bei *Vicia Cracca* L. Bot. Mag. Tokyo **28**:131–147. *pl. 2.* 1914.

is a partial alveolization and anastomosing of the chromosomes, but no complete resting stage. The second or homeotypic division follows very quickly.

Extranuclear nucleoli appear about the time of the second contraction, and are seen at all subsequent stages with the exception of the metaphases of the two mitoses. It is thus held to be not improbable that there is some connection between the substance in these bodies and that of the spindle.

The foregoing results are of interest in connection with the observations which the reviewer[2] has made on the nearly related *Vicia Faba*. In this species there is a very clear conjugation of leptonema threads before synizesis, many more stages in the somatic prophase, and a much more definite size difference in the chromosomes, both in somatic and in maturation mitoses, than SAKAMURA has shown in *Vicia Cracca*. These facts have led the reviewer to believe either that the latter species is not so favorable as an object for study as *Vicia Faba*, or that there are to be made out many more details than SAKAMURA has represented in his rather inadequate series of figures.—L. W. SHARP.

Sporogenesis in Equisetum.—In the third of a series of studies in spore development, BEER[3] gives an account of sporogenesis in *Equisetum arvense*. In the premeiotic cells the spirem, discontinuous from the first, arises from the reticulum by condensation and the breaking down of anastomoses along certain lines. The spirem segments appear to be unsplit until they become arranged on the spindle in the form of definite chromosomes. At telophase the chromosomes develop a reticulum by the formation of numerous anastomoses, but without internal vacuolation.

In the spore mother cell the discontinuous spirem is formed from the reticulum during synizesis. A longitudinal split visible during the early stages disappears as the spirem thickens. In a former note, BEER held this to represent parasynapsis, but as a result of the present study he has altered his opinion with regard to the origin of the heterotypic chromosomes. He now takes the view of FARMER, and believes they arise by the folding together of pairs of somatic chromosomes placed end to end in the spirem, the folding occurring at the time of the second contraction. During this period the nucleolus gives off material which appears to contribute to the growth of the chromatic threads.

The spindle is formed in the manner described by OSTERHOUT, with no centrosomes. The two maturation divisions occur as usual, but with an intervening resting stage. After the second division the resting reticulum is developed in the same manner as in somatic cells.—L. W. SHARP.

[2] SHARP, L. W., Somatic chromosomes in *Vicia*. La Cellule **29**:297–331. *pls. 2*. 1913.

———, Maturation in *Vicia*. (Prelim. note.) BOT. GAZ. **57**:531. 1914.

[3] BEER, R., Studies in spore development. III. The premeiotic and meiotic nuclear divisions of *Equisetum arvense*. Ann. Botany **27**:643–661. *pls. 51–53*. 1913.

Coal Measure plants.—Our knowledge of the flora of the British Coal Measures has been enriched by recent contributions from KIDSTON[4] and ARBER.[5] The former paper is devoted to a description of the plants secured from the beds cited in the title. Among the forms referred to the ferns, 20 genera are included, represented by about 60 species. New species are described under *Sphenopteris*, *Pecopteris*, and *Neuropteris;* while a new sporangium genus (*Coseleya*) is proposed, which may represent the microsporangium of one of the Cycadofilicales. Among the Equisetales, 7 genera are included, represented by 25 species, among which is a new species of *Palaeostachya*. The Lycopodiales are represented by 12 genera, including 34 species, among which are new species of *Sigillaria* and *Lepidocarpon*. There is also a new *Sphenophyllum*. The Cordaitales are represented by 9 species. Eight new seeds are described under *Samaropsis*, *Tripterospermum* (2), *Polypterospermum*, *Lagenostoma* (2), *Rhabdocarpus*, and *Hexagonocarpus*. A new species of *Whittleseya*, a genus usually referred to Ginkgoales, calls forth the opinion of the author that it is more related to Cycadales.

ARBER'S paper, as its title implies, is more concerned with the geology of the beds investigated, but it lists a large number of the plants that are made the basis of conclusions as to comparative stratigraphy. Among them two new species of *Sigillaria* are described.—J. M. C.

The embryo sac of Peperomia.—FISHER[6] has extended our knowledge of the embryo sac situation in *Peperomia* by a study of additional species, chiefly from Jamaica. The investigation included 8 species of *Peperomia* and also *Piper tuberculatum*. In all the species of *Peperomia* the mature sac is 16-nucleate, one nucleus maturing as the egg nucleus, another one as a synergid, 6–9 fusing to form the primary endosperm nucleus, and the remainder cut off individually by walls and later degenerating. In connection with the first two divisions of the mother cell, evanescent walls appear and reduction occurs, the conclusion being clear that the first 4 nuclei of the sac are megaspore nuclei. In *Piper* the mature sac is 8-nucleate, and is developed directly from the mother cell.

The author reaches the general conclusion that all of the peculiarities of the sac are derived, and that therefore the embryo sac of *Peperomia* does not represent a primitive condition.—J. M. C.

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[4] KIDSTON, R., On the fossil flora of Staffordshire coal fields. III. The fossil flora of the Westphalian series of the South Staffordshire coal fields. Trans. Roy. Soc. Edinburgh **50**:73–190. *pls. 5–16*. 1914.

[5] ARBER, A. E. NEWELL, On the fossil floras of the Wyre Forest, with special reference to the geology of the coal field and its relationships to the neighboring Coal Measure areas. Phil. Trans. Roy. Soc. London B **204**:363–445. *pls. 26–29*. 1914.

[6] FISHER, G. CLYDE, Seed development in the genus *Peperomia*. Bull. Torr. Bot. Club **41**:137–156, 221–241. *pls. 3–6*. 1914.

Fertilization of synergids.—Occasional anomalies have been noted even in 8-nucleate embryo sacs developed from a single megaspore. PERSIDSKI[7] describes additional cases in *Delphinium elatum*. Usually the development of the sac is normal, the egg having a vacuole at its micropylar end, while the synergids have the vacuole at the opposite end. In some cases the position of the vacuoles is reversed, so that the synergids have the organization of eggs and the egg has the appearance of a synergid. One case is figured in which the two male nuclei are fusing with the nuclei of two such synergids. It will be remembered that GUIGNARD figured two embryos of *Naias major* which may have arisen in this way. PERSIDSKI also figures an egg apparatus of five cells; three eggs and two synergids. This emphasizes what most of us have long believed, that the various nuclei of the sac are homologous and may replace each other in function.—CHARLES J. CHAMBERLAIN.

A new Araucarioxylon.—Dr. STOPES[8] has described a new *Araucarioxylon* (*A. novae-zelandii*) from the Cretaceous of New Zealand. It is described as new because it differs greatly from the more imperfect specimens of fossil araucarians hitherto recorded from that region. Its chief differential feature is the extreme development of the rows of thickened tracheids on either side of the rays, which are filled with large "resin-spools." By "resin-spools" is meant deposits of resin in the form of large disks opposite the middle of the pith rays, the lateral extensions of these disks running up and down the containing wall for some distance. The new species has also much more regular and strongly marked annual rings than usual among araucarians, which is held to be good evidence that New Zealand had well marked seasons during the Middle Cretaceous.—J. M. C.

Cases of suspended vitality.—BULLER and CAMERON[9] have recorded some remarkable cases of suspended vitality. They have shown that the "fruit bodies" of *Daedalea bicolor* can retain their vitality when dried, kept in the dark, and exposed to ordinary air at room temperatures, for at least seven and a half years; while those of *Schizophyllum commune* endured the same treatment for at least five years and seven months. The fruit bodies of the latter fungus, after previous drying by exposure to phosphorus pentoxide *in vacuo*, retained their vitality after being kept for 16.5 months in a vacuum at a pressure of not more than 0.1 mm. of mercury, in the dark at room temperatures;

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[7] PERSIDSKI, D., Einige Fälle anomaler Bildung des Embryosackes bei *Delphinium elatum*. Mém. Soc. Nat. Kiew **23**:97–112. *figs. 6.* 1914.

[8] STOPES, MARIE C., A new *Araucarioxylon* from New Zealand. Ann. Bot. **28**: 341–350. *figs. 3. pl. 20.* 1914.

[9] BULLER, A. H. REGINALD, and CAMERON, A. T., On the temporary suspension of vitality in the fruit bodies of certain Hymenomycetes. Trans. Roy. Soc. Canada **6**:73–78. 1912.

and these samé fruit bodies were found to retain their vitality when dried, kept *in vacuo*, and at a temperature of liquid air for three weeks.—J. M. C.

Seedling anatomy.—In continuing his investigations of the seedling anatomy of Sympetalae, LEE[10] has published an account of the Compositae, having examined about 50 species, well distributed through the tribes. The general conclusions are as follows: all seedlings are either diarch or tetrarch; variations in vascular anatomy occur not only in nearly related species, but in different individuals of the same species, the inference being that seedling anatomy "is of no value in questions of affinity"; the evolution of the vascular structures of seedlings is probably not an extremely slow process; tetrarchy and diarchy have probably been "interchanged" several times during the evolution of angiosperms; physiological factors are probably not sufficient to account for all the structures found in seedlings.—J. M. C.

The origin of Ascomycetes.—In a paper which reviews all the available data, approximately 100 papers being cited, DODGE[11] discusses the relationships of the red algae and the Ascomycetes. It is a very useful summary of our knowledge of the reproductive structures of these two groups, as well as a clearly presented argument in favor of the view that the Ascomycetes are a monophyletic group and have been derived from the red algae. The reproductive structures of the two groups are compared in detail, and the interesting transitions shown by *Collema* and *Ascobolus* are described.—J. M. C.

Flora of Panama.—STANDLEY[12] has issued the first of a series of papers preliminary to a flora of Panama. The present paper contains descriptions of some 40 new species from tropical America, which are distributed among 18 genera belonging to the Cyperaceae, Leguminosae, Gentianaceae, and Rubiaceae. Two new generic names are proposed, namely *Nothophlebia* and *Geocardia* (*Geophila* D. Don, not Berg.) of the Rubiaceae, and the following genera have been revised: *Sommera* (5), *Watsonamra* (11), and *Cobaea* (18).—J. M. GREENMAN.

Variation in Oenothera ovata.—Mrs. BRANDEGEE[13] has discovered that this Californian species of *Oenothera* has a remarkable range of variation. Apparently it is a plexus of "elementary species" quite as numerous as have been found in *O. Lamarckiana* and *O. biennis*.—J. M. C.

[10] LEE, E., Observations on the seedling anatomy of certain Sympetalae. II. Compositae. Ann. Botany **28**:303–329. *figs. 2.* 1914.

[11] DODGE, B. O., The morphological relationships of the Florideae and the Ascomycetes. Bull. Torr. Bot. Club **41**:157–202. *figs. 13.* 1914.

[12] STANDLEY, PAUL C., Studies of tropical American Phanerogams, no. 1. Contrib. U.S. Nat. Herb. **17**:427–458, *pls. 24–31.* 1914.

[13] BRANDEGEE, KATHARINE L., Variation in *Oenothera ovata*. Univ. Calif. Publ. Bot. **6**:41–50. *pls. 8, 9.* 1914.